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MANUAL OF METHODS IN AQUATIC ENVIRONMENT RESEARCH

Part 2 – Guidelines for the Use of Biological Accumulators in Marine Pollution Monitoring

with the cooperation of the
United Nations Environment Programme

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UNITED NATIONS ENVIRONMENT PROGRAMME

MANUAL OF METHODS IN AQUATIC ENVIRONMENT RESEARCH

Part 2 - Guidelines for the Use of Biological Accumulators in Marine Pollution Monitoring

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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PREPARATION OF THIS MANUAL

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Effects of Pollutants on Living Aquaric Resources and Scientific Basis for Monitoring

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PREFACE

At its Seventh Session in October 1973, the FAO Advisory Committee on Marine Resources Research (ACMRR) agreed to establish a Working Party charged with the task of examining "... the area of bioaccumulators in monitoring programmes to detect contaminants in the marine environment, especially at low level concentrations ...".

Under the co-operative project of the United Nations Environment Programme (UNEP), entitled "Effects of Pollutants on Living Aquatic Resources and Scientific Basis for Monitoring", the Working Party held two meetings in Rome (FAO Fish.Rep., 160 and 165). The first meeting was held in December 1974 and the second in July 1975 and prior to each meeting the members of the Working Party prepared documents dealing with particular aspects of the problem. At the first meeting the documentation was very much of a preliminary nature on which detailed discussion was based. At the second meeting the papers took the form of draft chapters on monitoring of groups of contaminants using biological organisms. Much of the second meeting was taken up verifying statements and ensuring that the views expressed in individual chapters were accepted by the Working Party as a whole.

This Manual includes the end products of those discussions, plus a Matrix Table produced as a corporate effort and intended as a quick means of indicating which organism might be suitable for monitoring a particular contaminant.

The Working Party was chaired by Dr. J.E. Portmann of the Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Burnham-on-Crouch, England, who was also responsible for general editorial duties related to the chapters. Final editing was carried out by staff of the Fishery Resources and Environment Division of FAO, particularly Dr. H. Naeve, who also acted as Technical Secretary of the Working Party.

The views expressed in the chapters are those of the individual authors and the Working Party and do not necessarily represent the views of either FAO or UNEP.

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1. THE ROLE OF BIOLOGICAL ACCUMULATORS IN MONITORING PROGRAMMES

by

J.E. Portmann

1.1 Introduction

This manual has been drawn up with the assumption that it is likely to be used in the design of a wide variety of programmes which would range in scale from ones with a purely local sphere of interest to those with a national, regional or even global coverage. A basic requirement of all such programmes is that they should be capable of establishing, and following, spatial and temporal variations in contaminant levels which occur in the sea, whether these result from man's activities or from natural action such as volcanic activity or leaching of metals from metalliferous ores or run-off from land.

Most pollutants can be expected to enter the sea via rivers and streams, municipal and industrial sewers, as a result of deliberate dumping or by aerial transport. Dredging and mineral extraction programmes may also introduce certain pollutants, especially metals. The relative importance of these various input sources differs according to the contaminant in question and the major sources are discussed in more detail in chapters 2 to 6.

1.2 Definitions

In studies of a local or national nature, there will usually be an intention to control input rates, should the levels revealed by initial measurements be considered dangerous to either man or marine organisms. Such an intention will also require information on rates of input of the compound or element in question and adds another dimension to the study. Most regional and global scale studies, as at present visualized, do not include a control element and have as their objective purely the indication of spatial and temporal trends. Regular measurement of contaminant levels was probably first conducted on radionuclides and it was found useful to distinguish between these two objectives. The lead taken by IAEA in this matter has been followed by some other international organizations, e.g. ICES, and the two types of programme are distinguished by describing the former as monitoring and the latter as surveillance. Such a distinction is not at present universally adopted and many so-called monitoring programmes merely involve surveillance; without any intention of denigrating the latter, a distinction between the two is often useful, e.g. in terms of cost-benefit analysis when frequency of observation is being debated.

Monitoring can be defined as the measurement of a pollutant or its effects with a view to the assessment or control of exposure to that pollutant of either man or specified elements of the biosphere. This type of operation is exemplified by the monitoring of deliberate discharges of radioactive materials to ensure that permitted levels are maintained and the discharge and predicted environmental levels are not exceeded and that predicted environmental levels are achieved.

It should be noted that this definition does not necessarily imply repeated measurement, since, if the programme is soundly based and initial measurements reliable, it could be that the first data obtained allow an assessment of no risk to man or any marine organism of interest, thereby rendering further measurement somewhat pointless except on a long-time scale - years, rather than months. Adequate control of the discharge being effected through the monitoring of discharge rates alone.

Surveillance on the other hand can be defined as the repeated measurement of a contaminant or its effects, with a view to establishing temporal or spatial trends in the levels or effects of that contaminant. In the light of assessment of the results of these measurements it is possible that controls will be considered necessary, and that the surveillance role will be upgraded to monitoring, possibly implying a greater frequency of observation. For example, levels of radionuclides arising as fallout from nuclear weapons testing are

kept under surveillance as part of a general programme of measurement of background radionuclide measurement. The results of such programmes are utilized in monitoring of discharge levels since background levels have to be accounted for.

It is advisable to recognize that, as implied by the difference in definitions, a distinction should be drawn between contaminants and pollutants.

Pollution, in a marine context, has been defined in many ways but, for the purpose of this manual, the definition developed by GESAMP has been adopted, i.e. Pollution is the introduction by man, either directly or indirectly, of substances or energy into the marine environment (including estuaries), resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities, including fishing, impairing the quality for use of sea water and reduction of amenities.

A pollutant is a substance which causes pollution and by definition must be having a harmful effect. Generally pollution in the marine environment, at least as presently recognized, is confined to the coastal margins of the land masses and, in general only occurs in restricted areas where input is heavy. There are a number of notable exceptions to this generalization, e.g. the alleged effect of DDT residues on the Bermuda Petrel, an ocean-living avian fish predator (Wurster and Wingate, 1968).

A contaminant on the other hand is a substance which would not normally be present in the marine environment, at least at the levels being found, but which apparently causes no ill effects. If the concentration increases with time there are likely to be harmful effects and the contaminant can rightly be classified as a pollutant. In most cases, especially those of a global nature, surveillance programmes will be concerned with measuring materials which at present are best described as contaminants not as pollutants.

However, in recognition of the fact that certain terms have a popular usage and for the sake of simplicity, throughout the remainder of this manual only the terms monitoring and contaminant will be used. It should be recognized therefore that monitoring includes surveillance and that sometimes a substance, although described as a contaminant, may be causing pollution under particular circumstances. These distinctions may at first sight appear academic but they are often useful in the detailed definition of particular programmes.

Finally, in relation to the subject of this manual, it is useful to define the term bioaccumulation. Bioaccumulation is that phenomenon which, regardless of reason and mechanism, results in a marine organism or particular tissue of that organism accumulating a higher concentration of a contaminant than that which is present in the sea. Since levels of most substances in the sea are low and their measurement poses great difficulties, this capacity of certain marine organisms has led to their adoption in marine monitoring programmes. Although in many instances it is possible to use chemical methods of concentrating the contaminant, there are several reasons why the use of a biological accumulator might be preferred. These are discussed below in general terms and, in relation to specific contaminants, in more detail in the appropriate sub-sections of the manual.

1.3 Bioaccumulation and Toxicity

The effects of contaminants on the biota are not discussed in this manual, which deals only with bioaccumulators as monitoring organisms. It is recognized, however, that the two subjects cannot be considered in isolation. A knowledge of the rates of bioaccumulation of chemicals, their routes of uptake and their subsequent internal distribution and metabolism is essential when designing toxicity experiments. It is also invaluable in the interpretation of their results and in extrapolating these data to the environment.

1.4 Reasons for Using Bioaccumulators

For most substances at present recognized as causing pollution, the levels in sea water are extremely low and as such their measurement poses great difficulties to the analytical chemist. In certain cases, e.g. radioactivity, the same problem may be faced in effluent discharges. In such situations the biological accumulator presents advantages in that it will concentrate the contaminant to a level which can more readily be measured. Since most monitoring programmes will involve a number of different laboratories, and since these will have different capabilities in terms of detection levels due to differences in analytical experience and instrumentation, it is advantageous to use biological accumulators as these will raise the detection levels required to an order attainable by most participants. Also, since sea water is not a homogeneous medium, it is useful to have some means of averaging out the variations which occur on a short-term temporal or spatial basis; a biological accumulator will achieve this.

A "synthetic animal" such as an ion-exchange resin may well perform many of these same functions but it is probable that an organism will discriminate between biologically available and non-available forms of the contaminant, whereas the "synthetic animal" may not take the same distinction. In addition, chemical analytical time is usually limited and has to be allocated according to the most effective use. Since data on contaminant levels in marine organisms is often also of relevance from a dietary intake standpoint, use of appropriately selected marine organisms may enable two purposes to be fulfilled by one sample and analysis.

In order to satisfy this sort of dual requirement, the monitoring programme will have to be carefully designed. Other design requirements would be that the scale of sampling in any one area be tailored to the degree of risk, either in terms of input levels or the size of the resource or population potentially at risk. Thus, the design of a monitoring programme would ideally follow an input survey. This may not be possible in other than broad terms, and the use of biological accumulators sampled in many different areas may well reveal unexpected sources of contamination. In general, differences revealed in levels of contaminants in biological accumulators will reflect differences in levels in the environment - a basic requirement of most global programmes as currently envisaged.

1.5 Difficulties Inherent in Using Bioaccumulators

There are certain difficulties involved in using biological accumulators in monitoring programmes. These are discussed in more detail in the sections which follow but it must be recognized that variations can occur according to the size, age, sex, etc., of the organism used. The condition of the animal may also be affected by the general quality of its environment and this, in turn, might affect either its uptake rate or saturation capacity. Thus, a lower level in an organism from one area relative to another may merely reflect a lower efficiency of accumulation. The animal operating at low efficiency may still be perfectly suitable for use in studies involving temporal changes rather than comparison of levels from place to place.

However, although a biological accumulator will in most cases shed its body burden of a contaminant if the level of contamination decreases, the measured level does not normally change dramatically. Nevertheless, in coastal and, especially, estuarine situations, a high level may merely indicate that a plug of highly contaminated water was present for a relatively short term, perhaps some time previous to sampling. In order to have detailed information as to when this occurred, water analysis would have to be undertaken, and use of biological accumulator would be on a first screen basis only, unless adequate information on uptake and loss rates had previously been obtained from laboratory studies.

1.6 Requirements of a Good Biological Accumulator

With such difficulties to be considered, it is clear that, for the analytical advantages to outweigh the disadvantages inherent in interpretation, the concentration in the animal relative to that in sea water (the concentration factor) must be considerable. An absolute minimum of 2 and preferably 3 orders of magnitude is necessary. If this condition cannot be met, there appears to be little advantage in analysing animals rather than water.

Since in an ideal situation it will be desirable to be able to link the level found in the animal with the level present in the water, it is essential that the uptake be affected by as few biological factors as possible and, preferably, uptake rates and plateaux should be linear with time and concentration respectively. The organism selected should preferably have a broad geographical distribution and where different species have to be utilized in neighbouring areas some overlapping is necessary. Ideally, the organism selected should be amenable to life under laboratory conditions to allow experimental data to be obtained on the relationship between levels and effects. Since most programmes will require repeated sampling and analysis, the organism selected should be available in appreciable numbers so that the monitoring programme does not decimate the population being sampled. For similar reasons it is also advisable not to design the programme based on a single species even though this may be the only one available throughout the study area.

Finally, the programme, whether designed essentially to perform a surveillance role or to fulfill a monitoring and control function, will have a basic requirement to provide an early warning of danger to man or the specified resource of interest. The organism must, therefore, reveal this danger well in advance of the danger actually occurring, i.e. well enough in advance for control measures to be taken, and have the desired effect of reducing input and hence reducing or at least stabilizing environmental exposure levels. In this context, a good example of biological accumulator use in monitoring programmes is the adoption of fish or shellfish as indicators of mercury pollution. Most marine fish and shellfish do not appear to be harmed by several tens of µg/g (wet weight) of mercury, although man is sensitive to only a few µg/g in his food. Mercury, in fact, is an excellent example since it is one of the few contaminants, which are recognized at present, for which reasonably accurate acceptable exposure criteria have been evaluated. The other major examples where these exist are for radioactive substances where again the major risk is to man and if he is protected so are marine organisms.

The need for adequately evaluated control criteria presents a major gap in present knowledge and, as a result, control measures are frequently imposed on a purely arbitrary basis with the result that complex effluent treatment plants have to be constructed and inputs reduced, perhaps without any real necessity. Where criteria of acceptable exposure limits are not yet available these should be established as a matter of urgency if monitoring programmes, such as global ones at present being discussed, are to be of any value. It should be recognized that, without such criteria, monitoring programmes will tend to degenerate into exercises which merely accumulate large amounts of numerical data, which may or may not be suitable for the establishment of trends. The conduct of such exercises is extremely wasteful in terms of manpower and valuable resources which could be much more gainfully employed in establishment of criteria. These criteria can only be derived from careful laboratory and field studies which are heavily dependent on analytical resources which are at present often employed on surveillance programmes.

A detailed discussion of the need for control criteria to be established or how this could be accomplished, is not appropriate to this manual but it is important that it be recognized that they are essential to the proper design of monitoring programmes. In this same context of providing the basic information required for the interpretation of results of monitoring programmes, it should be remembered that at least some information will also be required on contaminant concentrations in sediments and in water, and in other organisms, especially the target organism.

An essential adjunct to monitoring of levels of chemicals in the marine environment by the use of biological accumulators is the conduct of biological monitoring to determine the existence of pollution. Such studies are valuable since it is known that in a variety of polluted situations certain organisms do not survive, either because they or their progeny do not find conditions suitable; their absence may therefore indicate the existence of a pollution situation. It should be noted that in such a situation the absence of one or more marine organisms may be exploited by another opportunistic species which will then increase dramatically in number to the exclusion of other species. The number of materials which are at present recognized as causing pollution is not large and even if pollution is occurring and the pollutant is being accumulated by an indicator organism, the fact may go undetected owing to the absence of suitable analytical procedures. Biological surveillance may therefore provide an early indication of an unsuspected hazard. Once a biological effect has been observed, the analytical chemist has available a wide variety of powerful tools. With recent improvements in analytical diagnostic techniques, e.g. GC/MS, it is unlikely, once an effect is observed, that the causative agent will remain undetected for long.

1.7 Present Studies and the Lessons Learned

A number of contaminants are already the subject of routine programmes of analysis. For example, PCBs and organochlorine pesticides are measured in sea bird fat and eggs, fish and fish livers, heavy metals are measured in shellfish and radionuclides are measured in sea weeds such as Porphyra and Fucus. However, although the species chosen are excellent accumulators in terms of concentration factors, it must be recognized that, with the exception of radionuclide measurement, the usefulness of such activities is largely confined to the indication of changing levels in the physical environment, and the relationship between levels observed and measured effects is generally lacking.

The range of chemicals which can at present be monitored by use of biological accumulators is not large. However, it does embrace most of the substances which are known to be detrimental either indirectly to man or directly to one or more components of the marine ecosystem. It seems reasonable to assume that, as man relies more and more on synthetic chemicals for various purposes in his modern industrial and intensive agricultural society or in public health programmes, new substances will emerge which will also be amenable to monitoring or surveillance by the use of bioaccumulators. It should be noted that in most of the known cases, e.g. DDT, PCBs and mercury, our attention to danger signals was drawn to increasing levels in certain organisms because a species or community was showing signs of population decline or other severe stress.

This does not necessarily mean that the detection of a substance in an organism, at levels either above that found or detectable in the environment, is indicative of harm. Indeed a number of substances, e.g. phthallic acid esters and a few organosilicon compounds, can be detected in marine organisms but so far as can be established do not have any effect on the animal. In this context, it is perhaps worth noting that bioaccumulation can occur as a result of the animal storing the chemical in a non-biologically important site or form, e.g. cadmium granules stored by shrimps or lead stored in the exoskeleton of crustaceans. However, it should be recognized that, in some cases, this immobilization may be temporary and last only until the storage organ or tissue is utilized, e.g. DDT storage in the fat reserves of marine birds, fish and seals.

Most of the monitoring programmes at present in operation have been devised for purely national purposes and, in most cases, have been designed to provide trend type information plus, in some cases, data to form the basis of assessment of hazard to man or a marine resource. Under such situations, the measured level of a contaminant as opposed to the actual level was less important, since generally all the measurements for any one programme would be undertaken by a single laboratory. International programmes are now being developed and it is hoped that this manual will provide advice which will assist in the most effective conduct of such programmes. No international programme will be worthwhile if it is not carefully designed and incorporates appropriate arrangements for standardization and intercalibration, so that all laboratories provide actual level data.

1.8 Intercalibration

An essential feature of an international or even multi-laboratory national programme is the harmonization of sampling and analytical procedures. This should not necessarily be taken to mean standardization upon a single method to be used by all laboratories since this tends to prevent analytical development and improvements and does not necessarily guarantee identical results. In any case the use of a single method is rarely practicable in the light of differing degrees of instrumentation and analytical sophistication in the laboratories which will take part in international programmes. It is however essential that all analytical methods should be intercalibrated to ensure that any variations observed are not purely due to differences which arise during the analytical stage of the programme. The need for intercalibration and the manner in which it can be conducted is covered in more detail in chapters 7 and 8. There are, however, a wide variety of internationally available reference materials for a range of contaminants and substrates. Most of these have been analysed by a large number of workers and the true levels are fairly well established. It should, therefore, be possible to ensure analytical uniformity even though the problems of biological sampling may be more difficult to overcome. In any new study, it should be preferable to use existing intercalibration standards or reference materials rather than to devise new ones specially.

1.9 Species Identification

Wherever possible, it is desirable that clearly identifiable species be used to avoid confusion in interpretation of the results. However, whichever species are selected they must be clearly identified. It is not uncommon for a single species to be referred to by different laboratories under more than one specific name. Furthermore, many different species may, in fact, be referred to as a single species. No one laboratory nor any one country is likely to have the scientific expertise sufficient to resolve all of these systematic problems. Therefore, in order to ensure that each organism is correctly identified, systematic authorities should be designated to verify representative collections of their speciality. Sufficient funds will need to be made available to ensure that identifications can be verified rapidly.

1.10 Data Required in Reporting Results

Once the monitoring programme has been designed and the various tasks have been allocated to the laboratories concerned and the necessary intercalibration exercises have been conducted or are underway, the plan can be put into operation. Attention should, however, be given to the way in which results will be reported and with what detail. Experience has shown that many results have been rendered meaningless by failure to take elementary precautions in preparing sample material or to record vital information. Often at the time of reporting this information may appear meaningless but it is far better to record and report too much than too little. The information required for any sample should include at least the date and site of collection of the sample, the species and number of individuals which make up the sample, their weight, size, sex and condition. It is generally advisable to ensure that the organism or part thereof is free of other material which could lead to erroneous results, e.g. polychaetes and bivalves should, if possible, be allowed to cleanse themselves in clean water for 24 hours. Details as to whether or not such steps have been taken should be recorded and accompany the results. Details of the particular organ analysed and its weight individually and relative to the whole animal are also required. It is also desirable that the wet to dry weight ratio be given so that, regardless of whether the result is quoted in terms of wet or dry weight, both figures will be available. On the analytical side, it is necessary to report the details of sample storage, preparation and analytical methods including detection limits and the presence of any unexpected or unidentified substances.

From the foregoing it is clear that biological accumulators have a useful role to play in monitoring programmes and, as will be apparent from the following chapters, they may in some cases be almost essential in certain types of study. Certain problems are however attendant. It will be very easy to show differences, both temporal and spatial, but, in the light of the wide variety of habitat changes, it would be surprising if these did not occur.

In order to be able to understand and interpret these differences, the degree of abnormality of the changes must be assessed. For control purposes, there must be a relationship between the measured level and the measured effect. This requires an ability to establish the relationship between biological and environmental variables and the levels of contaminant measured by the analytical chemist. These are all areas which require further work. Nevertheless with careful design, monitoring on a local or global scale is possible at the present time. It must be recognized, however, that trends may be revealed which are the result of natural variables, not differences in contaminant levels, and that great care is necessary in the design of programmes and in the reporting of the results, if the results are to be as valuable as the cost of producing them would imply.

1.11 References

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2. MONITORING OF RADIONUCLIDES

by

J. Pentreath

2.1 Introduction

The levels of radionuclides in the marine environment have been enhanced as a result of man's activities over the last three decades. Nevertheless, of all the contaminants that have been deliberately introduced into the marine environment, that which has been most carefully calculated, monitored and controlled, is the waste arising from the peaceful use of nuclear power. As part of this controlled disposal, considerable use has been made of biological material, other than that which lies in a direct route to human exposure, to serve as an indicator of the presence of radionuclides in the environment. This experience has led to a number of general principles and practices which lend themselves, in part, to surveillance of other pollutants. It has also highlighted some problems which may arise.

2.2 Inputs and Routes of Entry

There is no zero level of radioactivity. The present background level has two principal sources: naturally occurring radionuclides and radionuclides resulting from weapon-test fallout. More than 60 radionuclides occur naturally. They are derived both from minerals of the earth's crust (e.g. ^{40}K, ^{226}Ra) and from cosmic ray activity (e.g. ^{3}H, ^{14}C). The most abundant radionuclide in the sea is ^{40}K, which occurs at a concentration of approximately 300 pCi/l in open sea water and accounts for more than 90% of the total radioactivity. The next most abundant natural radioisotope, in terms of radiometric units, is ^{87}Rb. Other radionuclides of interest are ^{238}U and ^{234}U which collectively account for approximately 3 pCi/l. Naturally occurring radionuclides are relatively well distributed in comparison to those arising from other sources.

The atmospheric testing of nuclear devices has, to date, contributed the largest fraction of artificial radioactivity to the environment (Joseph *et al.*, 1971; Preston *et al.*, 1972). Radioisotopes of particular interest from this source are ^{3}H, ^{137}Cs and ^{90}Sr. The distribution of fallout radionuclides is variable for a number of reasons (Mauchline and Templeton, 1964; Volchok *et al.*, 1971; Duursma, 1972). Where the explosion has taken place over the sea, a considerable degree of local contamination occurs. On a world-wide scale, there are differences due to the types of nuclear devices tested, and to their altitudes when detonated. Latitudinal zonation occurs as a result of currents in the upper atmosphere, and there are also variations in wash-out as a result of local precipitation, particularly along coast lines.

In addition to the above sources of radioactivity, the marine environment receives radionuclides introduced deliberately, and in a controlled manner, as waste arising from the operation of nuclear power installations and nuclear fuel reprocessing plants. Such operations result in a variable degree of local enhancement of a number of radionuclides. The local distribution of such radionuclides is dependent upon prevailing tidal and current patterns, and the nature of nearshore bottom deposits which serve as sites of adsorption. Although it is impossible to generalize on their distributions, Table I gives an indication of the levels of some radionuclides, from all three sources, which could have been expected along a sampling line toward the open sea at Windscale, U.K., in 1973. As can be seen, the nearshore levels of ^{40}K are slightly less than those of open sea water, due to dilution from land run-off. This run-off, however, slightly elevates radionuclides arising from weapon-test fallout. The fall off in levels of discharged radioactivity is rapid, although some radionuclides, such as ^{137}Cs, are more conservative in sea water than, for example, ^{106}Ru. The levels of all discharged radionuclides may also be higher, at equal distances from the discharge point, in the direction of prevailing water movements along the coast.

Table I

The concentrations of radionuclides (pCi/l) which could have been expected along a sampling line toward the open sea at Windscale (U.K.) in 1973

Sampling position	5 miles	50 miles	150 miles
Salinity	34.0°/oo	34.5°/oo	35.0°/oo
Natural radionuclides			
^{3}H	≈3	≈3	≈3
^{40}K	260	270	280
$^{234}U + ^{238}U$	≈2	≈2	≈2
Fallout radionuclides			
^{3}H	50	≈30	≈10
^{90}Sr	0.2	0.1	0.1
^{137}Cs	0.3	0.2	0.1
^{239}Pu	0.001	<0.001	<0.001
Discharged radionuclides			
^{3}H	450	50	≈10
^{90}Sr	50	5	0.5
^{137}Cs	200	15	2
^{106}Ru	75	2.5	< 2.5
^{239}Pu	0.5	0.015	≈0.001

Other, more occasional, sources of radioactivity may be enumerated. Radionuclides used as tracers in a variety of scientific and industrial disciplines may ultimately reach the sea as a result of disposal under authorization. In addition, accidental release may occur as a result of the loss of nuclear weapons, nuclear powered vessels and radioisotopic power generators used in aerospace vehicles such as the SNAP and RIPPLE devices (Joseph et al., 1971).

2.3 Analytical Methods

The nuclei of some isotopes are not stable but disintegrate spontaneously at a characteristic rate of decay. Such unstable isotopes are called radioisotopes, or radionuclides. Radionuclides emit particles, and/or photons, of which the following are used in radiometric analyses: alpha particles, beta particles, gamma-ray photons and X-ray photons. The energy units used for radiation measurement are multiples of the electron volt (eV), which is equivalent to the kinetic energy acquired by an electron on being accelerated through a potential difference of one volt. Radiations interact with all matter causing ionizations and excitations which may then produce chemical changes. It is these effects which are utilized in the various methods of detection and measurement. Decay schemes and methodology can be found in standard texts (e.g. Birks, 1964; Siegbahn, 1965; Lederer et al., 1967).

2.3.1 Sample preparation

Most environmental samples are subject to some form of preparation although, for many radionuclides, it may be possible to obtain an immediate result by the analysis of samples in a wet state. Normally, however, samples are treated to remove excess water, either by freeze-drying or, less frequently, by oven drying. Such treatment reduces the sample bulk, thus allowing an improvement in counting geometry. Samples are not usually ashed, except where a chemical separation is necessary, as for alpha emitters and certain radionuclides such as ^{55}Fe. Water samples are prepared either by drying or the use of precise techniques to extract one or more elements. A review of recommended methods for marine environmental samples has been published by the International Atomic Energy Agency (1970).

2.3.2 Radiometrics

Detector systems differ in their complexity. Total alpha, beta or gamma counting can be made. For environmental surveillance, frequent use has been made of the total beta method to detect abnormal variations (Mitchell, 1967).

The individual estimation of gamma emitters is achieved by spectrometry of the characteristic energy spectrum of the emitted radiation. Complex mixtures can be analysed, although it is sometimes necessary to make an initial chemical separation into sub-groups of radionuclides. Such a practice is usual for low concentrations of radionuclides which have coincidental gamma energy peaks. Two commonly used detector systems are sodium iodide (thallium-activated) crystals and lithium-drifted germanium detectors. The latter give superior resolutions, for the crystal sizes available, but display poorer sensitivity. For example, they allow the resolution of such mixtures as ^{95}Zr, with 0.724 and 0.757 MeV peaks, and the 0.766 MeV ^{95}Nb peak, which are otherwise indistinguishable, and not readily resolvable from the 0.796 MeV ^{134}Cs peak, which might also be present (Fig. 1). Such resolution circumvents the otherwise tedious chemistry required. A high degree of data processing, of varying sophistication, is needed to resolve complex spectra.

Total beta activity measurements are frequently used. Two systems are generally employed: "infinite depth" counting and the "thin source" technique. In the former method samples are presented to the detector and their count rates compared with a suitable standard. The method is limited, however, since it discriminates against low-energy beta emitters in comparison to a ^{40}K standard. But such discrimination can be useful and has, for example, provided a simple check for the presence of ^{106}Ru in marine samples (Mitchell, 1967, 1968). This method also presents problems resulting from variations in packing density and geometry. The second technique requires that the sample be prepared so as to present as thin a source as possible, to reduce self-absorption, to the detector. This method is also not without disadvantages, however, since the use of a thin end-window for the detector increases its sensitivity to alpha emissions. A gas-flow detector is therefore required, which by operating in the proportional region can distinguish between such emissions by pulse-height discrimination. Thin source methods are generally less sensitive and require more complex equipment.

Those radionuclides which emit beta particles only, for example ^{90}Sr, ^{32}P and ^{63}Ni, require suitable chemical separation. They are then counted either by one of the above methods or by liquid scintillation.

The measurement of total alpha content presents similar problems to those of beta counting. Samples are usually ashed, to reduce their bulk, and counted using a zinc sulphide scintillation screen. For more detailed analysis isotopes are chemically separated and counted using spectrometric methods, usually with silicon surface barrier layer detectors.

Finally, although many decay processes involve X-ray emission, it is not usually necessary to resort to this form of detection. An important exception is ^{55}Fe. Following chemical separation the ^{55}Fe is determined either by proportional counting, after electroplating on to a copper disc, or by gel scintillation counting.

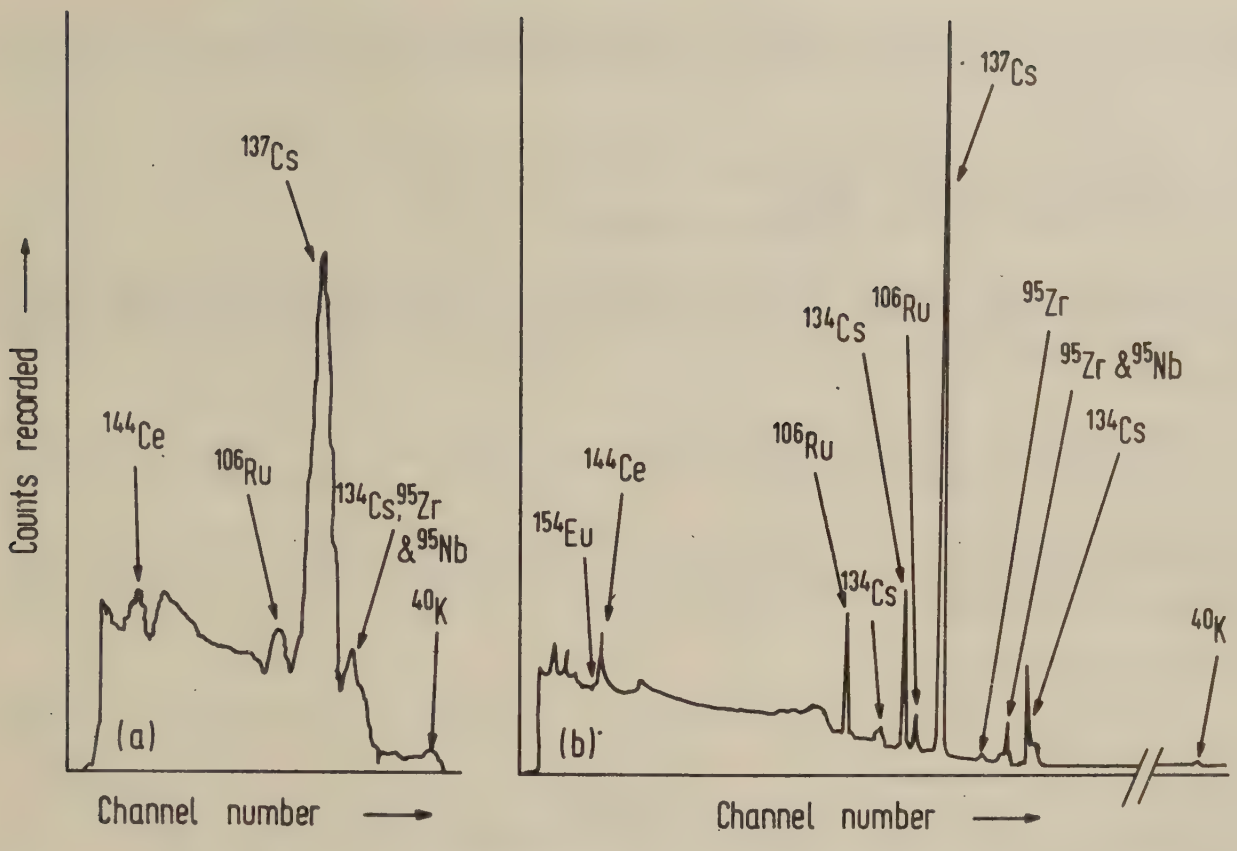

Figure 1. Gamma spectra of a sample of Fucus vesiculosus collected near Windscale, U.K., (a) using a NaI(Tl) crystal and 200 channels and (b) using a Ge(Li) detector and 2000 channels

2.4 Bioaccumulation

2.4.1 General aspects

The ability of the biota to concentrate a large number of trace elements over the ambient levels of sea water has long been noted. There is, therefore, a strong reason for considering biological samples as indicators of radioactivity; although, since inorganic material such as mud and sand have a high affinity for many trace elements, these should also be considered. Indeed, marine sediments display concentration factors (pCi g^{-1} sample/ pCi g^{-1} water) in the range of 10^4 for a large number of radionuclides. Biological materials are usually preferred, however, since for comparative purposes sediments differ considerably in their geochemistry from one site to another, and their activities, and specific activities (pCi g^{-1} of element), will be different at different depths within the sample taken.

One may initially consider whether there is any advantage in taking a biological sample rather than a direct sample of the sea water. It is possible to measure a number of fission product nuclides, such as ^{137}Cs, ^{144}Ce, ^{106}Ru, $^{95}Zr/^{95}Nb$ in 5-10 l of sea water at a concentration of 1 pCi/l. At levels <1 pCi/l it is relatively easy to pass the sea water through a resin to collect ^{137}Cs, which has a low concentration factor in the biota. For the other radionuclides, however, it would be necessary to take much larger water samples, directly proportional in volume to the reduced level. The use of a natural concentrator of these nuclides is therefore one of immediate practical advantage.

Biological accumulation is a process of adsorption, absorption or both. In their simplest forms rates of accumulation of single elements may either be regarded as linear, i.e.

$$C_t = It + C_o \tag{1}$$

where C_t = concentration in the organisms at time t
I = rate of intake, e.g. pCi/g/t
and C_o = concentration in the organism at time to

Such an equation is the most simple approximation to a net uptake, which continues until the saturation value has been attained. Or, alternatively, it is frequently observed that an element is both accumulated and excreted until an asymptotic, or steady state, value has been attained, i.e.

$$\frac{dC_t}{dt} = I - KC_t \tag{2}$$

where K = the excretion coefficient

Equation (2) integrates to

$$C_t = \frac{I}{K} (1 - e^{-Kt}) \tag{3}$$

more commonly expressed as

$$C_t = C_{ss} (1 - e^{-Kt}) \tag{4}$$

where C_{ss} = asymptotic value

The value of K can be used to determine the biological half-life ($tb\frac{1}{2}$), since $tb\frac{1}{2} = \frac{\ln 2}{K} = \frac{0.693}{K}$

These equations imply a steady gravimetric level of the element in the medium. Such equations can only be regarded as approximations. It is frequently found that, even where an exponential form of turnover is broadly applicable, the observed data conform more precisely to a multi-exponential process. This is most clearly defined, experimentally, by following the loss of a tracer in unlabelled water. Thus

$$C_t = C_{o_1} e^{-k_1 t} + C_{o_2} e^{-k_2 t} + \ldots\ldots\ldots C_{o_n} e^{-k_n t} \tag{5}$$

Such experiments are usually influenced by the degree of success in labelling the pools which collectively constitute the body burden.

In radioecological studies, it is important to consider that the radionuclide itself undergoes physical decay. The environmental situation may frequently approximate to two extremes. One where the discharge can be considered to be fairly constant, and secondly where the contamination occurs as a single event. In the former, under approximate steady state conditions, the turnover of the radionuclide by the biota may be described by

$$\frac{dC_t}{dt} = K C_{ss} - (K + \lambda) C_t \tag{6}$$

where λ is the decay constant of the radionuclide, with a physical half-life ($tp\frac{1}{2}$) of $\frac{0.693}{\lambda}$

Therefore

$$C_t = \frac{K}{K + \lambda} C_{ss} (1 - e^{-(K + \lambda)t}) \tag{7}$$

If this is expressed as a concentration factor, $\left[C_t/W_t\right]$ where W_t is the concentration in the water,

$$\left[C_t\right] = \frac{K}{K+\lambda}\left[C_{ss}\right]\ (1 - e^{-(K+\lambda)t}) \qquad (8)$$

The value $(K+\lambda)$, $(\frac{0.693}{tb_{\frac{1}{2}}} + \frac{0.693}{tp_{\frac{1}{2}}})$ is equivalent to $\frac{0.693}{te_{\frac{1}{2}}}$,

where $te_{\frac{1}{2}}$ is the effective half-time, being a combination of the two "elimination" processes. The three values are related by:

$$te_{\frac{1}{2}} = \frac{tb_{\frac{1}{2}} \times tp_{\frac{1}{2}}}{tb_{\frac{1}{2}} + tp_{\frac{1}{2}}} \qquad (9)$$

The relative values of these three half-times determine the extent to which a radionuclide will be concentrated by any organism.

Where radioactive contamination occurs as a single event, the physical decay cancels out. This is the condition under which most experiments to determine kinetic values are made. Thus, the concentration of the radionuclides in the sea water may be described by

$$W_t = W_o e^{-\lambda t} \qquad (10)$$

where W_t = concentration in the water at time t
and W_o = concentration in the water at t_o

The concentration in the organism is therefore

$$C_t = C_{ss}\ (e^{-\lambda t} - e^{-(K+\lambda)t}) \qquad (11)$$

Dividing (11) through by (10) gives, as a concentration factor

$$\left[C_t\right] = \left[C_{ss}\right]\ (1 - e^{-Kt}) \qquad (12)$$

In practice the sea water concentration in the environment will fall faster than a rate due to λ alone because of variable dilution as a result of water mixing, precipitation, adsorption onto particulate material both in suspension and as bottom deposits - in addition to any removal by biological activity.

The range of concentration factors which can be expected is considerable. Information may be drawn from a large number of publications, largely based on stable element determinations. There have been several recent summaries (Mauchline and Templeton, 1964; Polikarpov, 1966; Bowen, 1966, Lowman et al., 1971). It is important to note that the term "concentration factor" can be misunderstood. The term represents a ratio, a ratio of the amount of element, or radionuclide, per unit weight of sample relative to the amount per unit weight of water. It does not mean that this ratio was necessarily derived by directed exchange between the two, but simply that, as a result of many metabolic processes, it is the ratio which is ultimately derived. Its precision is therefore also dependent on these metabolic processes and can rarely be defined within narrow limits. Similarly, it should be noted that the term "biological half-life" can be misinterpreted. As shown in equation (5), it is frequently observed that a single value is insufficient. In addition, in poikilotherms the half-life may be markedly affected by temperature, and also by body size, such that different half-times exist in the environment at different latitudes, different seasons and between individuals of the same population. Data which are given should, therefore, only be interpreted within the biological and physical boundaries prevailing when the observations were made.

The determination of the route of uptake of the radionuclides is clearly one of importance, and has been the subject of a number of laboratory studies. Algae appear to accumulate elements directly from the water and this, coupled with the sessile nature of the macrophytic forms, has led to their frequent choice as indicator material. For heterotrophic organisms, however, the relative roles of food and water should be individually defined. Limited information exists on the magnitude of these two routes for a number of elements. In general, the majority of radionuclides have been found to be principally accumulated via the food chain. Differences in individual body burden values may therefore be related to dietary anomalies.

Living organisms have a dynamic relationship with their environment and are thus affected by changes in temperature, light, salinity and pH. All of these have discrete effects on accumulation, but also affect rates of excretion, if excretion occurs, and may thus cancel each other out in the long term. Such factors do, however, make comparisons of samples, taken at different seasons, difficult to interpret. It is frequently found, for example, that one of the greatest obstacles to intercomparability is that of body size. The total element content can often be related to body size by a power function relationship such that

$$Y = aW^b \tag{13}$$

where Y = total element content and a and b are constants
and W = weight

Since, by dividing through by W, the concentration, Y^1 may be expressed as

$$Y^1 = aW^b - 1 \tag{14}$$

the relationship dictates whether the concentration will increase, decrease, or remain constant with growth. For example, where $b \approx 0.8$, as has been established for many physiological parameters such as respiration, the concentration will fall with a slope of -0.2. When $b = 1$ the concentration remains constant, and when $b > 1$ the concentration will increase with weight. Such relationships have been demonstrated for some metals in both fish and molluscs (Boyden, 1974). Similar power function relationships may be expected for temperature, salinity and pH, within the tolerated range, although examples are few (Pentreath, 1971).

An important aspect is the rate of change of body size, i.e. growth. This has been particularly studied in algae, since the concentration factor attained by different parts of the plant depends on their dry to wet weight ratios, which, in turn, differ from old to new thallus. In controlled experimental conditions it is difficult to maintain healthy algae without growth taking place. It is therefore necessary to incorporate an equation which allows for this increase in weight. Young (1975), has attempted to predict, from experimentally derived data, the changes in concentration factor for ^{65}Zn in a typical fucoid at different rates of growth (Fig. 2). The figure also shows that, under conditions similar to those described by equation (6), a difference in specific activity will occur, as a result of the interplay of the biological half-life of zinc and the effective half-life of ^{65}Zn.

In addition to what may be regarded as effects caused by metabolic processes, the accumulation of radionuclides can also be markedly affected by differences in the physico-chemical form of the radionuclide. Such effects have long been noted, and appreciated, but there is as yet little chemical information which is of immediate practical benefit to the biologist. The physical chemistry of sea water is complex; indeed, the physical chemistry of dissolved chemical species, in any solvent, at the very low gravimetric levels associated with radionuclides in the marine environment, is very little understood. It has been established, however, for a number of radionuclides, that differences exist not only between a radionuclide and the stable element, but between radionuclides deriving from local, coastal, disposal and fallout radionuclides in the open ocean. The effects of these differences on bioaccumulation have been reviewed by Robertson (1971). Radionuclides which have been studied include ^{65}Zn, ^{55}Fe and ^{106}Ru. Several studies have been made on ^{106}Ru and laboratory experiments have clearly demonstrated different rates of accumulation of different forms.

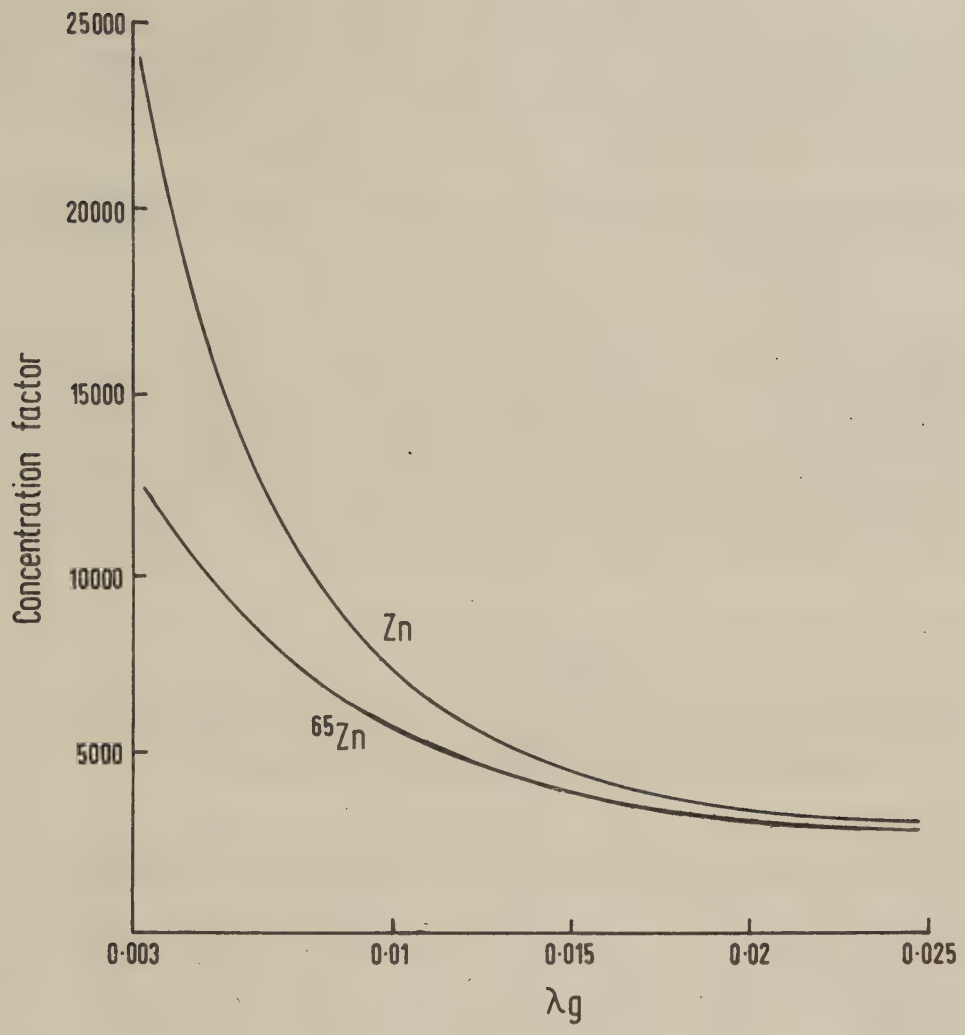

Figure 2. Theoretical concentration factors of Zn and ^{65}Zn in Fucus serratus growing at different rates. λg = growth rate constant/day (after Young, 1975)

It is not always appreciated that, for the majority of radionuclides, the gravimetric quantities of the element are extremely low. For example, 1 pCi/l of ^{65}Zn is only 1.2×10^{-10} μg/l of Zn. Variations in the levels of the stable element may therefore affect the rate of accumulation of the radionuclide. Laboratory experiments are frequently difficult to interpret as a result of a lack in their design to allow for both metabolic and physico-chemical effects (International Atomic Energy Agency, 1975), although an appreciation of them is essential to fully interpret the environmentally derived data.

2.4.2 Specific examples

Environmental monitoring programmes are directed primarily to the assessment of public radiation exposure. The various types of programmes used, and their basic philosophies have been the subject of numerous publications. Some recent reviews have been made by Preston

(1969), Mitchell (1974) and Mitchell et al. (1974). The selection of biological material in such programmes is normally based either on the extent to which they contribute to public exposure, i.e. the critical materials, or as indicator materials where contamination is below the limits of detection in the critical materials. Biological material has also been used to ascertain the retention of radionuclides in specific water masses, and to determine the limits at which contaminated water could be detected.

The basic requirements of a good biological indicator have been discussed in chapter 1. For radionuclides the greatest experience has been gained from the use of algae, molluscs and fish.

Algae. One alga which has received considerable attention, due to it serving not only as an indicator but because it is a critical material in one site evaluation, is the rhodophycean Porphyra /umbilicalis/. The radionuclide of interest is ^{106}Ru which is discharged in the area of Windscale, U.K., a fuel reprocessing plant. Jones (1960) has shown that the accumulation of nitrosyl ^{106}Ru by living and killed discs of Porphyra is very similar and that accumulation could be well described by the Freundlich adsorption isotherm. Since the accumulation was pH dependent, being suppressed at lower pH values, it was considered that cation exchange reactions were involved at the algal surface. The algae contain a sulphated polysaccharide which is essentially a chain of galactose units with $-OSO_3H$ groups at frequent,

Table II

Radionuclides in algae (in the pCi/g wet range) at St. Bees, Windscale, U.K., normalized to a Porphyra content of 1
(From unpublished material of D.F. Jefferies)

Species	^{106}Ru	$^{95}Zr/^{95}Nb$	^{144}Ce	^{137}Cs
RHODOPHYCEAE				
Porphyra /umbilicalis/ 1/	1.0	1.0	1.0	1.0
Rhodymenia palmata	0.9	2.6	2.7	7.4
Chondrus crispus	0.8	1.8	3.2	2.4
CHLOROPHYCEAE				
Enteromorpha spp.	1.0	5.0	4.6	6.2
Cladophora spp.	2.5	13.4	11.3	4.5
Ulva lactuca	1.8	10.3	6.9	5.1
Chaetomorpha spp.	1.2	8.0	6.1	8.9
PHAEOPHYCEAE				
Fucus spiralis	0.1	1.3	0.9	3.4
Fucus vesiculosus	0.2	1.6	0.8	3.9
Ascophyllum nodosum	0.1	1.0	0.5	2.8
Fucus serratus	0.4	1.9	1.2	3.6
Laminaria digitata	0.2	1.2	0.6	3.2

1/ For Porphyra the concentration factors are: ^{106}Ru, x1500; $^{95}Zr/^{95}Nb$, x400; ^{144}Ce, x1000; ^{137}C , x10

but irregular intervals (Wilson, 1968). In laboratory studies with the nitrosyl form, ^{106}Ru was adsorbed by the thalli of Laminaria digitata and Ulva lactuca greater than Porphyra laciniata (Jones, 1960). In the environment, however, the values for L. digitata have been found to be lower than those for Porphyra (Table II). It should also be noted that errors in measurement of environmental algae can result from the strong adherence of sediment to the surface of some species.

Samples of Porphyra taken weekly, near the discharge point, do not closely follow the quantities discharged (Fig. 3). The relationship will depend on physical processes within the near-shore water mass, but it is also likely to be influenced by seasonal differential growth. As can be seen from the figure, the algae have a cyclical variation in their dry to wet weight ratio. Nevertheless, the integrated accumulation has shown a variation in concentration of no more than about three to one, relative to the discharge, when followed over a decade of observations (Preston and Jefferies, 1969). The alga has also been used as an indicator of the dispersion of ^{106}Ru, and $^{95}Zr/^{95}Nb$, along the shore line (Fig. 4). Samples of both Porphyra and sea water, however, have demonstrated differences in the concentration factor of ^{106}Ru with increasing distance from the discharge point. Near the pipeline the concentration over water was approximately 1800, but fell to 600 within a distance of 20 miles (Fig. 5). Such differences may arise from different physico-chemical forms of the radionuclide possessing different partition coefficients in sea water.

Other radionuclides released from the Windscale plant are detectable in algae, and the concentrations differ considerably from species to species. Table II gives the concentrations in different algae relative to those in Porphyra. The genus Fucus is used regularly in the Windscale area for the detection of ^{90}Sr and ^{110m}Ag. In fact, the routine analysis of Fucus has revealed the presence of ^{99}Tc in the area (Mitchell, 1973). This radionuclide is conservative to sea water, concentrates in Fucus species, but has not been detected in Porphyra.

Since the Fucus genus has a high concentration factor for many neutron activation products, as well as for the fission product nuclides, it has been used as an indicator at a number of nuclear power station sites. At Hinkley Point (U.K.) traces of ^{65}Zn, ^{55}Fe and ^{60}Co have been detected in F. vesiculosus but none has been detected in the potentially critical materials (Mitchell, 1969). Such data can be used to make a quantitative estimate of exposure via the critical materials if the relative concentration factors are known. A discussion on the possible use of other species to detect a number of radionuclides in the vicinity of nuclear installations is given by Zattera and Bernhard (1970).

The mechanisms of metal accumulation by algae have been the subject of several studies. Those on the accumulation of ^{65}Zn by Gutknecht (1961, 1963, 1965) showed that uptake was largely non-metabolic. Alginic and other insoluble acids were suggested as agents for the exchange process. The accumulation of ^{65}Zn has also been studied by Bryan (1969). This study, with Laminaria, demonstrated differences which can occur in specific activity values, since the laminae adsorbed relatively more zinc from low, than from high, sea water concentrations. Higher zinc concentrations were also attained by slow-growing weed because it had a lower weight/unit area. The presence of other metals was found to have an effect. If the concentrations of manganese or cadmium were raised, the adsorption of ^{65}Zn could be slowed, or even stopped. Such effects were reversible, but a similar effect of increased copper in the water was not. The accumulation of both ^{65}Zn and ^{59}Fe by Fucus serratus has been studied by Young (1975), who showed that ^{65}Zn uptake was a net uptake process (equation (1)). Experiments with ^{59}Fe, however, showed that some exchange did take place.

Attention has also been paid to the use of algae as indicators of plutonium. Wong et al. (1971, 1972) have shown that concentrations vary widely in the environment and that samples should, if possible, be normalized with reference to surface areas.

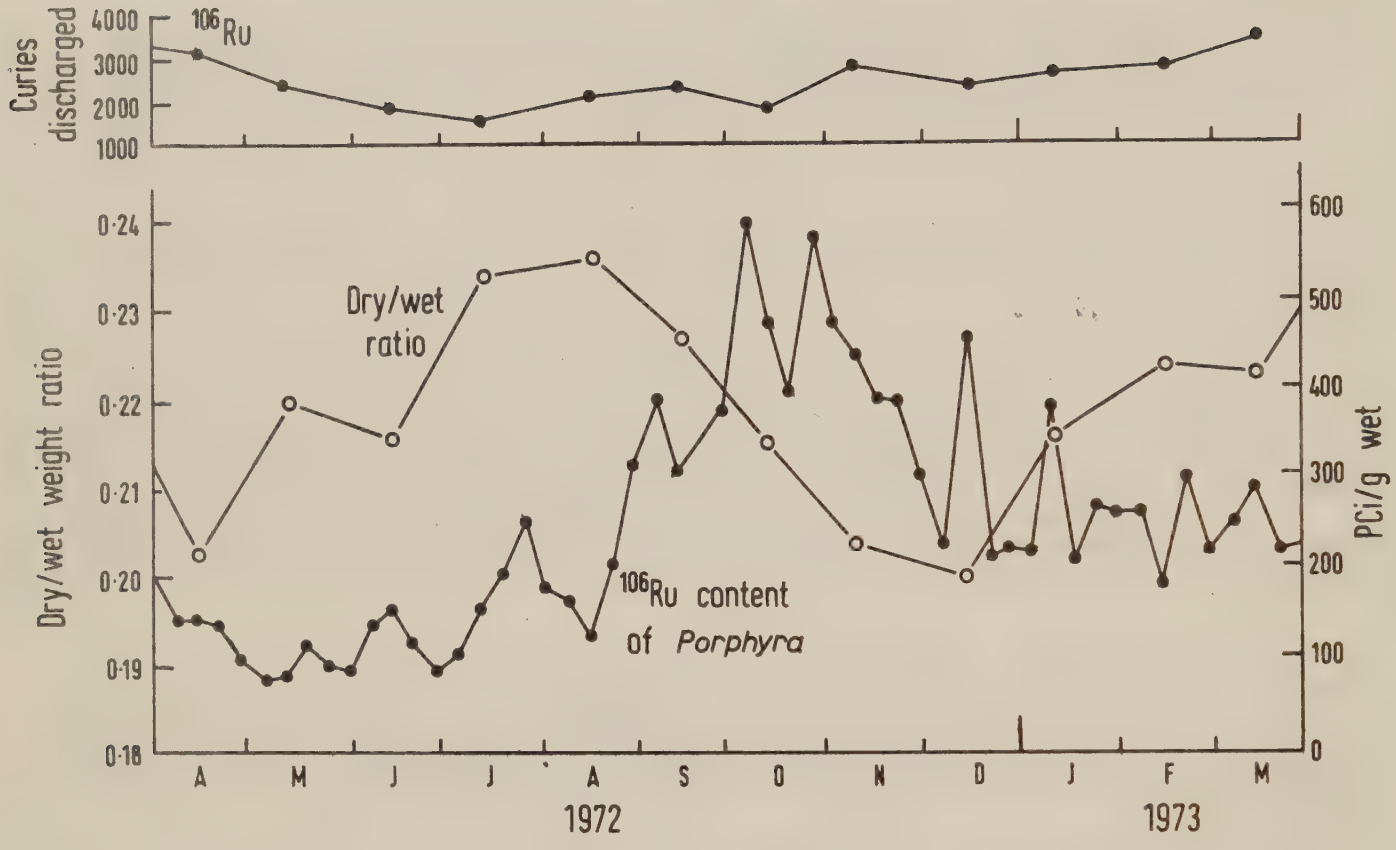

Figure 3. Variations in the ^{106}Ru concentrations of Porphyra (umbilicalis) at Windscale, U.K., in relation to the amount of ^{106}Ru discharged and the dry to wet weight ratios of the alga

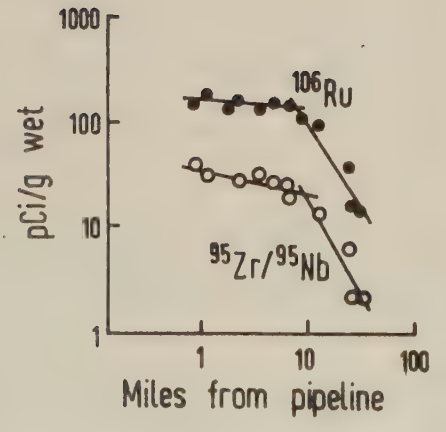

Figure 4. Radionuclides in Porphyra as a function of distance from Windscale, U.K., 1964-66 (after Preston and Jefferies, 1969)

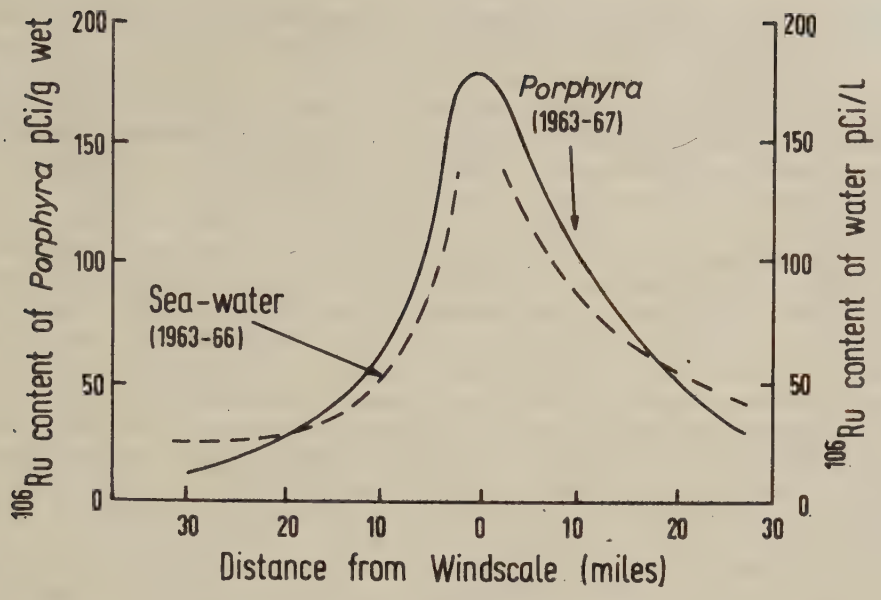

Figure 5. The concentration of ^{106}Ru in Porphyra and sea water as a function of distance from Windscale, U.K., 1963-67 (after Preston and Dutton, 1968)

In a study using the giant kelp, Macrocystis pyrifera, Hodge et al. (1974) warned that for comparative studies only blades of the same age should be compared. This is a very fast growing species, however, and samples of Porphyra on the Windscale coast have shown a relatively constant relation to the amounts discharged over a number of years (Hetherington et al., 1975). As with all biological indicators, their value is frequently one of either detecting the presence or absence of a radionuclide, or providing an integrated accumulation over a longer time scale, rather than one of providing very precise reflections of environmental change.

Molluscs. Although algae have many advantages as indicator organisms, it must be noted that they are limited to the euphotic zone, and are usually taken from the littoral zone. They are also not present in all localities, and are not suitable for all elements. Some heterotrophs have therefore been examined with a view to their use as indicators of contamination. There are many disadvantages. In particular, there is the problem of the relative roles of food and water, and, for bivalve molluscs, that of the degree of contamination, both internally and externally, by sediment. Bivalves should be allowed to depurate before analysis.

There are a number of papers on the levels of various elements in the tissues of molluscs, and their concentration factors over water have, with other biotic groups, been frequently summarized (e.g. Lowman et al., 1971). Some of the more useful species are lamellibranchs such as oysters, mussels, clams and some of the gastropods. It is known that considerable differences occur, not only in the concentrations in different species, but within different

tissues of the same species (e.g. Brooks and Rumsby, 1965; Segar et al., 1971). It is also known that the rates of accumulation of radionuclides differ from one organ to another, as do percentage turnover and retention times. For example, in a lamellibranch such as Mytilus the total element flux of a number of neutron activation products is particularly dictated by those of the stomach and digestive gland (hepatopancreas) and by the gill (Pentreath, 1973). Thus, the relative sizes of these organs, which vary in individuals due to allometric growth, makes it difficult to compare "total soft part" values.

There is evidence for marked seasonal variation in the trace element content within a species, although the number of studies is limited. Bryan (1973), studying eleven elements in two species of lamellibranchs, found higher values in autumn and winter. Such variations were thought to be related to seasonal food supply. It was suggested, however, that both digestive gland and kidney had potential as indicator organs. A similar example was provided by Romeril (1974), who studied a number of metals, in two lamellibranchs, in an estuarine area. A considerable range of values was found at different times of the year and in different age groups. The levels were also related to the degree of local water turbidity and, for some metals, to the concentrations in the sediment. As with algae, the rates of accumulation of one isotope can also be affected by the presence of other metals (e.g. Romeril, 1971).

Lamellibranchs have nevertheless been frequently used as indicators. In a number of studies on the Atlantic coast of the U.S.A. a variety of species have been used to detect fallout radionuclides. Wolfe and Schelske (1969) detected higher levels of a number of nuclides, particularly $^{95}Zr/^{95}Nb$ and $^{140}Ba/^{140}La$, within a short period of atmospheric weapons testing. It was also found that concentrations and retention periods differed from one sampling area to another within an estuary, in relation to different salinity regimes. More detailed studies, both in the laboratory and in the field (Wolfe, 1967; Wolfe and Coburn, 1970; Wolfe, 1971) have shown that ^{137}Cs concentrations in Rangia cuneata varied inversely with salinity and directly with temperature, and thus also varied with season. In contrast, ^{106}Ru and ^{55}Fe were found at higher concentrations in more saline waters (Wolfe and Jennings, 1973). Investigations with other species have shown the value of species specificity for particular radionuclides such as ^{54}Mn in the scallop, Argopecten irradians (Schelske, 1973; Schelske et al., 1973) and ^{65}Zn in the oyster Crassostrea virginica (Wolfe, 1970; Schelske et al., 1973).

Lamellibranchs have also been used as indicators close to sources of contamination such as the Hanford reactors in the U.S.A. A number of publications exist on their value for detecting radionuclides at distance from the source (e.g. Young and Folsom, 1973; Larsen et al., 1973) and to monitor the response in environmental concentrations when disposal has ceased (Seymour and Nelson, 1973). In some areas lamellibranchs are the critical materials for discharge control, such as ^{65}Zn and ^{110m}Ag in oysters at Bradwell in U.K. (Preston, 1966, 1968; Preston et al., 1968). Gastropods have also been used as indicators of specific radionuclides such as ^{110m}Ag, ^{58}Co and ^{60}Co (Patel et al., 1973; Folsom and Hodge, 1974). In the majority of these studies, the precise relationships between the concentrations in the biota and the environment are not known but the data have been sufficient to generalize on the distribution of the radionuclides, and to control discharges effectively.

Fish. More limited use has been made of vertebrates as indicators of radioactivity. Fish are mobile. It has been shown that their retention of a number of fission product nuclides, with the exception of some of the monovalent elements, is poor (Chipman, 1960; Jones, 1960; Mauchline and Taylor, 1964; Pentreath and Jefferies, 1971). The accumulation of ^{134}Cs and ^{137}Cs has been studied in some detail for one benthic species (Morgan, 1964; Jefferies and Hewett, 1971; Pentreath and Jefferies, 1971), and it has been shown that even for this monovalent element food accounts for a large fraction of the intake, and may reflect dietary preferences. For other radionuclides, particularly those derived from neutron activation, the main route of accumulation is via the food chain (Pentreath, 1973). It has been shown in a number of studies that concentrations of many elements vary both with size and age, and that regulatory abilities exist. However, since many fish have a comparatively

long life-span they can integrate many elements over a longer time scale, although this will depend on the effective half-life. In addition, for large water masses, they are the only suitable biota to monitor. A number of studies have therefore been made.

Folsom et al. (1969) attempted to correlate the concentrations of caesium in Thunnus germo* and those in the surface waters of the Pacific. Estimates were made using values derived both from the natural ^{133}Cs and fallout ^{137}Cs. Further studies were made on ^{54}Mn, ^{60}Co, ^{65}Zn and ^{110m}Ag in T. germo livers (Folsom et al., 1973). Data obtained from ^{65}Zn measurements indicated an enhancement from the Columbia river along the Californian coast. The ratio of ^{65}Zn to ^{60}Co was also taken as an indication of enhancement as a result of atmospheric weapons testing. Repeated observations (Hodge et al., 1973) have shown that many radionuclides may be retained in the biota of the upper water masses in the Pacific for a decade or more.

By comparing fish species Folsom and Hodge (1974) have shown that the ratios of radionuclides may be of greater importance than absolute values. For example, Thunnus albacores caught in the mid Pacific had higher concentrations of ^{110m}Ag in their livers than T. alalunga caught closer to the San Onofre nuclear power plant. The former species, however, had ^{108}Ag : ^{110m}Ag ratios near unity, whereas the latter had up to 17 x more ^{110m}Ag in some samples. These ratios were even more marked in invertebrates and algae taken near the power plant. Differences in the ratios of nuclides (e.g. $^{65}Zn/^{54}Mn$) in the viscera of Pacific salmon have also been noted from fish taken to the north and south of the Columbia river (Kujala et al., 1969). Concentrations of radionuclides were found to be related to the different trophic levels of the different species in their diets.

2.5 Assessment of Results

Concern about the impact of radioactivity on the marine environment is such that all deliberate discharges to it are monitored in advance. Even in accidental situations an estimate can usually be made of the nature and quantity of the radionuclides involved. Biological material is therefore analysed either to ensure that the degree of public exposure is within the prescribed limits set by the International Commission on Radiobiological Protection (ICRP), or to serve as an indicator of the dispersion of the nuclides. Biological sampling can be of greater service. By definition it indicates those elements which can be accumulated and those which cannot. It also serves to inform of any possible environmental effect which could, or has, altered an element's biological availability, as in the classic example of mercury.

The principal choice of biological material being its importance as a pathway to human exposure, seafoods caught near a nuclear installation are regularly monitored, special attention being paid to the defined "critical" materials. Serious contamination of some substances can be immediately detected by hand-held instruments in certain circumstances, and samples can be rapidly analysed in laboratories (International Atomic Energy Agency, 1965, 1971).

For more general surveillance, other suitable indicators are chosen. In comparative programmes, consideration should be given to the ubiquity of the species and to its ecological niche. Such factors are important for intertidal species which, although similar, may experience considerable differences in emersion/immersion times. Different species of the same genus frequently occupy different intertidal zones, such as the fucoids quoted in Table II. Some species on the lowest part of the shore are constantly immersed but not readily collectable. Other species, although exposed when the tide ebbs, may be left in standing water or dry out considerably on hot summer days.

The frequency of sample collection should reflect the known discharge rate, its composition, the local patterns of dilution and dispersion and the extent of concentration by inorganic and biological material (e.g. Mitchell, 1967). Any sampling programme should also be planned with due consideration of the chosen population to withstand constant cropping.

(*) = Thunnus alalunga

Particular attention should be paid to the dangers of over-collection of less numerous invertebrate species, such as some of the molluscs; it would be ironic if the greatest environmental impact were to be the local decimation of a species collected as part of a programme designed to estimate environmental impact.

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3. MONITORING OF TRACE ELEMENTS OTHER THAN RADIONUCLIDES

by

E. Mandelli

3.1 Introduction

Contamination of the marine environment by trace elements, such as mercury, lead, cadmium, arsenic, copper, zinc and silver, is difficult to assess inasmuch as the natural levels of these materials is not well known. Despite this limitation, metal pollution has been clearly identified in many coastal areas of the world's oceans. In some cases unusually high inputs of toxic trace elements to the marine environment have resulted in ecological changes and financial losses to commercial fisheries and, in isolated cases, have even been hazardous to human health.

High "trace element" input into estuarine and coastal areas from effluent discharges, dumping and river runoff, have been well established. Addition of trace elements to the open ocean is believed to occur mainly through atmospheric transport from metropolitan and industrial areas. At the present time, measurement of concentration gradients from known input sites is the primary method of ascertaining trace element contamination of the marine environment.

Uptake and accumulation of trace elements by marine organisms has been invaluable in indicating environmentally enhanced concentrations of these elements. Although elevated levels of toxic trace elements in marine organisms are associated with known contaminated areas, little is known about the environmental and physiological processes that regulate the concentrations of trace metals in marine organisms. Moreover, their precise relationship to water levels is generally not well understood.

3.2 Inputs

Potentially toxic trace elements which normally appear in sea water as minor constituents are introduced to the marine environment by natural sources and as byproducts of various human activities.

3.2.1 Natural sources

Most of the natural inorganic compounds which reach the sea, both in solution and as suspended solids, are carried by rivers. Aerosols and weathering constitute the natural sources of elements dissolved in streams. On the continents aerosols have a significant "continental" component compared to the maritime component. It is not possible to state with any accuracy the exact contribution from weathering of most trace elements, compared to aerosol fluxes. Several estimates have, however, been made of the quantity of elements carried to the sea by the air (Bertine and Goldberg, 1971). Most trace elements are effectively adsorbed on particles (inorganic and organic) carried by streams. This results in the maintenance of fairly constant values of dissolved trace elements in the major streams of the world. Even industrial inputs of trace elements are effectively obscured by this effect (Turekian, 1971).

3.2.2 Man-made sources

Man's activities have increased the quantity of biologically available toxic trace elements in the marine environment. Diffuse discharges related to urbanization and technical development in general, together with point discharges from specific industrial activities, have been contributory factors.

Among the multiple pollutant sources introducing sizeable amounts of trace elements in the marine habitat, the following industrial activities appear to be the most important:

(i) Metal mining industries

(ii) Ferrous and non-ferrous metal industries, including metal plating

(iii) Industries producing both organic and inorganic chemicals

(iv) Offshore dumping of domestic sewage, sludges and various industrial wastes.

3.3 Trace Elements in the Marine Environment

Once trace elements have reached the marine environment, it is important to understand their distribution, dispersion, transformation and accumulation in the various interfaces. For a proper understanding of bioaccumulation mechanisms, as these elements migrate through the marine environment, a knowledge is needed of the chemical speciation and reactions they undergo.

3.3.1 Physical and chemical distribution

The flux of trace elements in the marine environment, particularly in estuarine and coastal areas, is controlled by processes that include adsorption-desorption reactions, flocculation, precipitation and sedimentation.

The dispersion of trace elements in the marine environment will be affected by properties, such as their volatility, tendency to form insoluble complexes with inorganic and organic compounds, and their ability to form either soluble complexes with ions common in sea water or oxides and sulphides of low solubility. Within the marine ecosystems, relatively high concentrations of trace elements are found in the air-sea surface, sediment-sea water and sea water-organism interfaces.

Particular trace elements, especially heavy metals, are concentrated in the sea surface microlayer by transport on particle associated bubbles (Duce et al., 1972). Despite injection of trace elements by streams, whether natural or artificial, since most of the input becomes trapped in the sediments and incorporated mainly into organic matter, most of the cationic forms have very little chance of leaving an estuarine or coastal area in solution. Trapping of trace elements also occurs through passive and active removal by organisms.

Chemical speciation of trace elements in natural sea water is a complex and highly variable process. Most dissolved trace metals can coordinate with a large number of naturally-occurring dissolved ligands (Zirino and Yamamoto, 1972; Elder, 1975). Because the identity of the predominant complexes changes markedly under different conditions, the diversity of dissolved trace element species is usually rather limited at any instant in a natural aquatic system, but becomes more complicated in time. Few generalizations can be made about trace metals in the aquatic environment, as they are highly dependent on the pH and anionic strength of the medium, thus suggesting that only certain species of a given trace element are available for bioaccumulation from sea water. It is possible to infer that ionic species are readily available to marine organisms, though little is known about competing complexation reactions at the sea water-tissue interface.

3.3.2 Biological transfer and transformation

Although biological transfer of trace elements through the food chain is of predominant interest, more information is needed to determine how modes of uptake, metabolism, storage and excretion influence their transport in the marine biota. Many of the differences in

trace element concentration found between species appear to be due to trophic level relationships. However, although food web magnification of trace elements has generally been an accepted premise in the past, there is evidence which suggests that trophic accumulation is but one of the factors affecting the transference of trace elements in marine organisms.

Transfer and transformation of trace elements can be brought about by plytoplankton and bacteria, due to their relatively large surface area to volume ratio, and also by seagrasses; in certain areas, these processes have been shown to be important. In addition to the ability to transfer trace elements, bacteria also have the ability to methylate elements such as mercury, and thereby mobilize elements trapped in the sediment.

The seagrass system is unique in that it absorbs trace elements from sediments, as well as from sea water. In this way metals that would otherwise be lost to the sediments can be returned to the food web by these rooted plants (Windom, 1974).

A further aspect of the biological uptake, retention and translocation of trace elements in members of the marine biota is related to chemical changes and storage tissues within the organism. Methyl mercury compounds, assimilated from water and/or through metabolic conversion of mercury, are accumulated in the muscle tissue of carnivorous fish, whereas ionic mercury is accumulated in their liver and spleen (Stickney et al., 1974). Cadmium, unlike mercury, does not appear to concentrate in fish flesh, but does accumulate in the gills, liver and GI tract (Eisler, 1974). Moreover, arsenic in marine fishes appears to be in the form of organic-arsenic compounds (Braman and Foreback, 1973). Changes in transfer mechanisms of trace elements, as related to the age and reproduction of the organism, must also be considered.

The kinetic aspects of trace element accumulation have been discussed in Chapter 2 of this Manual. It should be noted that radioisotopes have been used in many studies on the accumulation and retention of trace elements (e.g. mercury, cadmium and arsenic) and that many radionuclides are in fact metals of interest in the marine contamination field.

3.4 Accumulation of Trace Elements by Marine Organisms

There are numerous publications on the levels of trace elements in tissues of different biotic groups and their concentration factors over water levels (Lowman et al., 1971). There has also been considerable discussion in recent years concerning the applicability and feasibility of using marine organisms as indicators of trace metal pollution. From field surveys and laboratory experiments it has been recognized that exposure of a wide variety of marine organisms to relatively high levels of trace elements, results in concentrations in their tissues several orders of magnitude above the level in the water.

3.4.1 Environmental and biological factors

A knowledge of the route of uptake is a main factor in the interpretation of data from monitoring trace elements in the marine habitat. Autotrophic organisms appear to accumulate trace elements directly from sea water; thus, environmental parameters, such as temperature, salinity, light and pH; and chemical factors, such as speciation and competing complexation of the elements are of considerable importance. The presence of several potentially toxic trace elements can also affect their preferential uptake.

For heterotrophs, the routes of food and water must be individually defined, though limited information exists concerning the relative magnitude of these two routes. In general, the majority of trace elements have been found to be accumulated via the food chain. Differences in the values of body burdens of the various species may be related to diet (Topping, 1973; Hardisty et al., 1974). There is evidence of seasonal variations in the trace element content within species (Bryan, 1973), and of individual variations and considerable differences according to size and age (Cross et al., 1973). It is also known that accumulation of trace elements by different species varies from one organ to another,

as do turnover and retention time (Pentreath, 1973). The relative size of these organs, which varies with individuals, renders the comparison of total soft part values difficult. For some of the most useful species, the sessile organisms, internal and external contamination with sediments, can give rise to misleading accumulation values. Bioaccumulation of trace elements by mobile species, including fishes with limited range, is not readily interpretable (Portmann, 1972).

3.4.2 Uptake and loss of trace elements by marine organisms

Several mechanisms have been proposed for uptake and loss of trace elements by marine organisms, particularly heavy metals. Romeril (1971) summarized the following uptake pathways:

(a) Adsorption of ions at membrane-water interfaces

(b) Adsorption by active and/or passive diffusion of metal ions from sea water across semi-permeable membrane into the body fluids

(c) Ingestion of ions with food or in combination with particulate matter and absorption through the gut wall.

Bryan (1971) proposed two mechanisms for the loss of heavy metals from biological material:

(a) Excretion across the body surface or gills

(b) Excretion via the gut and the urine.

Living organisms have a dynamic relationship with their environment; therefore, it is frequently observed that a trace element is both accumulated and excreted until steady state has been attained. Both short and relatively long term experiments, using bivalves, appear to indicate that rates of uptake for trace elements are proportional to their concentration in the medium (Pringle et al., 1968; Majori and Petronio, 1973). Rates of loss are correlated to internal concentration of the element (Schulz-Baldes, 1974).

Where the rate of accumulation is linear, the determination of an upper saturation level is particularly important. If the environmental concentration of a particular toxic trace element persists over a sufficient period of time, the organism may become affected physiologically, so that it is no longer useful in assessing environmental changes. An additional compliçation arises when the regulating capacity of the organism allows it to maintain a nearly constant tissue burden, within a range of trace element concentrations. Patterns of regulation are also complicated, and relatively constant levels may be maintained in some tissues at the expense of others. Direct relationships with the environment may be obscured by slow response times for short-term variations of trace elements in water and tissues.

3.5 Criteria for Selection of Bioaccumulators for Trace Elements

It is suggested that for marine organisms to be considered useful bioaccumulators of trace elements for monitoring purposes they should possess certain characteristics. The species to be selected should be representative of various stages within the food web and should also be sessile or have a limited range of movement. When large water masses are to be compared, mobile species (fish, birds, mammals) should also be considered. It is assumed that any indicator organism selected should have an affinity for the element under study.

3.5.1 Summary of data by element and organism

A comprehensive summary of the information available up to 1950, concerning major and minor elements in marine organisms, was published by Vinogradov (1953). This work provides extensive data on the elemental composition of marine algae, bacteria, higher aquatic plants, protozoa, porifera, coelenterata, bryozoa, brachiopoda, annelida, echinodermata, mollusca, crustacea, tunicata and fishes. Thirtytwo elements are discussed, including most of the trace elements potentially harmful to living resources and hazardous to human health. Since Vinogradov's review, a considerable amount of additional information has been gathered on trace element accumulation by marine organisms.

More recent works by Riley and Segar (1970) and Segar et al. (1971) present data on major and minor trace element concentrations in several species of echinodermata, coelenterata and mollusca. Eisler (1973) has compiled 567 references on the biological effects of metals to aquatic organisms indexed by element and taxa.

The IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN Joint Group of Expert on the Scientific Aspects of Marine Pollution (1974) has compiled brief reviews on a number of elements which have been considered as potential pollutants. A list of 15 elements is given: mercury, cadmium, lead, copper, zinc, arsenic, antimony, beryllium, chromium, cobalt, manganese, nickel, selenium, silver and vanadium. The brief reviews on each element include information on main sources, method of production, uses and effects, especially on marine life. However, information on the accumulation of trace elements is restricted to a few phyla of marine organisms, and covers only a few elements, mainly, copper, zinc, iron and manganese.

Sessile organisms have successfully been used to monitor trace element accumulation in the marine biota. Benthic algae have received considerable attention, particularly in the surveillance of aquatic environments for radionuclides, as well as for stable elements (Zattera and Bernhard, 1970). In this connexion, rhodophycean and phaeophycean algae, Porphyra, Fucus and Laminaria, have been studied for their ability to act as indicators of trace element contamination (Bryan, 1969). Bryan and Hummerstone (1973), working with Fucus vesiculosus from estuaries, concluded that, for long-term indication of trace element contamination, a reasonable reflection of the average concentration of these elements could be obtained if samples were taken from the same position at the same time of year. Seasonal changes in zinc, copper, cobalt, iron and cadmium content in both Fucus vesiculosus and F. serratus were observed by Fuge and James (1974). Preston (1973) also used macrophytes, to show differences in large areas of water around the U.K. and detected zones of high metal contamination. Among the most frequently used organisms for monitoring purposes are sessile molluscs, such as oysters, mussels, clams and limpets. Probably one of the most complete works on trace elements in marine bivalves was published by Brooks and Rumsby (1965). They discussed variations in the accumulation of trace elements in Ostrea sinuata, Pecten novae-zelandiae and Mytilus edulis and within their different tissues. Pringle et al. (1968) reported on accumulation of copper, zinc, lead and cadmium under laboratory conditions, by the soft shell clam Mya arenaria, the oyster Crassostrea virginica and the hard shell clam Mercenaria mercenaria. The results of this study showed that for any given metal and set of experimental conditions, the uptake rate and tissue concentration level decreased in the organisms in the above species order.

Other studies by Bryan (1973) and Romeril (1974) relate to the accumulation of trace elements by scallops. Both workers report finding a broad range of values at different times of the year and in different age groups. Since mussels display a distinct ability to accumulate heavy metals, species of the genus Mytilus have been widely used, perhaps because of their ubiquity. Details on the use of Mytilus for trace element monitoring programmes can be found in the works of Preston et al. (1972), Majori and Petronio (1973) and Schulz-Baldes (1973, 1974).

Crustaceans have been used as bioaccumulators to a lesser extent since they appear to be able to regulate their trace element body burden. For example, in some crustaceans the levels of zinc in their blood and muscle tissue can be regulated by increasing the load in the hepatopancreas and the urine (Bryan, 1964). Eisler et al. (1972) studied the accumulation of cadmium by Homarus americanus under laboratory conditions and found that relatively high levels of cadmium occurred in gills and viscera. Topping (1973a) reported on the concentration of copper, zinc, cadmium and lead in the lobster Homarus vulgaris and the crab Cancer pagurus collected from coastal areas of Scotland.

More limited use has been made of marine vertebrates which take up many potentially toxic trace elements via the food chain, resulting in a lowering of their concentration at higher trophic levels (Pentreath, 1973a). Where fish samples from different locations have been analysed (Portmann, 1972; Topping, 1973; Windom et al., 1973; LeBlanc and Jackson, 1973), the results are not easy to correlate with environmental levels. Many metals appear to be regulated by vertebrates which inhibit formation of a linear relationship to the levels in the environment.

The results of a study conducted by Cross et al. (1973) indicate that the accumulation pattern of trace elements in fish muscle can vary as a function of the species of fish, its size, and the element investigated. The maintenance of similar concentrations of elements such as manganese, iron, copper and zinc in muscle tissue throughout the life-span of a fish suggests that, except for the trace elements accumulated by new tissues, the exchange of metals between muscle and blood are essentially constant.

In the particular case of mercury, it appears for many fish that muscle tissue never achieves a steady state. Portmann (1972) observed that, in general terms, mercury accumulated in fish tissue was consistent with expected differences in the areas from which the fish had been sampled.

Bioaccumulation of heavy metals by marine phytoplankton has been reported by Morris (1971) and Preston et al. (1972). Knauer and Martin (1973) reported seasonal variations of cadmium, copper, manganese, lead and zinc levels for surface phytoplankton samples in Monterey Bay, California, U.S.A. This variability was related to the composition of the standing crop, the biomass and the presence of organic detritus during upwelling periods. Mercury levels in naturally occurring phytoplankton and zooplankton in Monterey Bay were also studied by Knauer and Martin (1972). On a dry weight basis, mercury levels in phytoplankton were about twice those found in zooplankton (copepods and euphausids). However, as in the case of the bioaccumulation of other heavy metals, mercury concentrations by phytoplankters was highly variable, not showing any meaningful seasonal trend. This wide fluctuation in the concentration of mercury in phytoplankton was in direct contrast to the remarkable constant mercury levels in zooplankton.

Phytoplankters are probably not suitable organisms as indicators of trace element contamination, since accumulation factors vary considerably with environmental conditions and species composition. Also sampling techniques do not discriminate between phytoplankters and other organisms and materials such as microzooplankton and organic particulates are known to concentrate trace elements in the sea surface microlayer (Wallace and Duce, 1975).

3.5.2 Role of bioaccumulators in assessing toxic trace elements

The passage of potentially harmful pollutants through the marine reservoir, until they are removed to a sink, is variable in time scale. During this variable period, long-term effects on the biological components of the world's coastal and oceanic areas are possible, as a result of recurrent exposure to toxic materials. In this connexion, baseline studies on bioaccumulators of potentially toxic trace elements are essential in order to assess natural levels in marine organisms. This information will also be valuable in establishing the biological storage component for the mass balance of trace elements in aquatic ecosystems.

It should be stated that, although surveillance and monitoring of trace elements through bioaccumulators may appear to be a simple operation, this is not the case. As discussed in previous sections, trends displayed by organisms, such as regulation, retention and translocations within organisms, may be affected by many natural variables. Consequently, the precise relationship between bioaccumulators and toxic elements to the water levels are not well understood but it is doubtful that any simple relationship exists. Analysis of trace elements in accumulator organisms seems likely to provide comparative, rather than absolute, information.

3.6 Analytical Procedures

A brief outline on the analytical procedures used in the determination of potentially toxic trace elements in biological systems will be given here. Further details are given in Chapter 9 and in Part 1 of this Manual (FAO, 1975).

3.6.1 Sampling recommendations

The sampling procedure must be capable of providing a representative sample. An important part of the sampling procedure is the recording of complete information about conditions in which samples were collected. Biological samples should, as a general rule, be kept deep frozen between collection and laboratory treatment. Not very much is known about changes that occur in the concentration of trace elements when comparing tissues frozen some time after collection of the organisms to that of immediately frozen samples.

Treatment of samples before freezing will vary according to the organisms; for example, macroalgae should be washed, preferably in water from the same environment, to remove sediment material, and the sample to be analysed should not include other attached organisms. Smaller invertebrates should also be washed and then placed in borosilicate glass or polypropylene jars and kept deep frozen. Filter-feeding animals, such as bivalve molluscs, and burrowing animals, such as polychaetes, should be allowed to self cleanse of ingested sedimentary material before preservation.

Subsequent treatment of bivalves consists of opening the shells with plastic knives, pouring off the water and washing particles from inside. After the water has been removed, the shells should be closed again before being placed in containers for deep freezing. It is easier to use larger animals, such as fishes. Each fish of the catch should be washed and put into plastic bags and deep frozen.

Not very precise rules have been established regarding the biomass to be sampled. For fish samples, a number of species should be collected and, if possible, 10 to 15 individuals of each species should be selected, each of the same age or length. It is advisable to sample more than one year class. For bivalves, it is convenient to sample between 25 and 50 specimens of each species and size group.

3.6.2 Analytical techniques including preparation of samples

When analysing biological samples, the choice of tissues is determined by the aim of the analysis. It is important that in each case the same type of tissue should be analysed for all specimens of the same species. For analysis of heavy metals in fish, liver and kidney are good test materials. In the case of invertebrates, which are often too small, whole body tissues are normally used. If tissue samples from several specimens are to be used, it is important that the same weight of tissue be taken from each of the individual specimens.

It is necessary for some organisms, especially invertebrates, to determine the water content of the sample in order to calculate the contaminant level found on a dry weight basis.

Homogenization of composite samples can be accomplished with stainless steel homogenizers. For trace element analysis, it is desirable to freeze dry the samples followed by homogenization in a glass mortar. From the homogenate, a subsample should be used, of a size selected according to the sensitivity of the analysis and the expected concentration of the contaminant in the sample. Sample sizes corresponding to at least 5-10 g wet material are often necessary for determination of less abundant trace elements.

Organic matter is digested by oxidative treatment. Usually one of the following methods are used: wet oxidation, dry oxidation, or fusing oxidation. Details of these techniques are given by Gorsuch (1970).

Contamination of samples with impurities from the reagent or from storage vessels is frequently a serious problem. High quality reagents are necessary and glass apparatus used should be pretreated. It is important that blank determination be carried out through the whole analytical process.

The most widely used methods for the analysis of trace elements are Atomic Absorption Spectrophotometry, Emission Spectrometry, Flame Emission Photometry, Activation Analysis, Gas-liquid Chromatography and Electrochemical Analysis. For a more comprehensive discussion of these techniques, the reader is referred to the recent reviews published by Lisk (1974) and FAO (1975).

3.7 Reporting of Results

The data should be quantitative to permit rapid evaluation. A descriptive list can be developed to include the data derived from analysis and logistic data, corresponding to each sample. The complete data for each sample should be coded in the appropriate format to be punched on computer cards. Executive command control systems, such as ENVIR, developed by Gulf Universities Research Consortium, Galveston, Texas, can be conveniently used. (Menzies, 1973). This particular system has great flexibility for increasing or decreasing coded descriptions. The information is stored on tapes, which can be utilized with different computer programme modules. Initially, analysis of the data is best conducted on a local basis with eventual broad regional expansion.

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4. MONITORING OF PETROLEUM HYDROCARBONS

by

R.F. Lee

4.1 Inputs

There is little question that millions of tons of petroleum find their way into the sea each year. The sources of this petroleum include marine transportation, offshore oil production and coastal oil refineries, sewage outfalls, natural seeps and atmospheric fall-out. Published estimates of the sources and quantities of these various inputs are available in a recent National Academy of Science Report (Ocean Affairs Board, 1975). The largest contributor (approximately 2 million tons/year) is marine transportation, which includes losses during normal ship operations, oil spills resulting from accidents at sea and spills during terminal operations.

4.2 Analytical Methods

The basic analytical procedures for the isolation and identification of petroleum hydrocarbons in marine organisms may be separated into four steps (see Figure 1). Further discussion of these methods, including advantages and disadvantages of particular methods, is presented in part 1 of this manual (Carlberg, 1975).

(i) Sample collection without contamination.

(ii) Extraction of lipid by use of solvents such as methanol or benzene.

(iii) Separation of petroleum hydrocarbons from other lipids by chromatography (thin layer, column chromatography, high pressure liquid).

(iv) Identification and interpretation of the data obtained by some method, such as fluorescence spectrometry, gas chromatography or gas chromatography-mass spectrometry.

Besides hydrocarbons, there are also nitrogen, oxygen and sulphur containing compounds in petroleum which may enter marine organisms. The fate of these compounds, including the possibility of bioaccumulation, is at present unknown.

4.3 Biological Transfer and Transformation Processes - Distribution

4.3.1 Uptake

Petroleum can enter the marine food web by adsorption to particles, followed by ingestion of the particles by filter feeding, by the active uptake of dissolved or dispersed petroleum and/or passage into the gut of animals that gulp or drink water. The results of analyses of marine organisms exposed to oil spills has demonstrated their ability to take up and store hydrocarbons, without necessarily indicating the mode of uptake (Blumer and Sass, 1972; Burns and Teal, 1973; Clark et al., 1973; Farrington and Quinn, 1973; Zitko, 1971). Large amounts of tar were found in the stomach of three sauries collected in the Mediterranean Sea (Horn et al., 1970). Fish caught in water near petrochemical industries often have a kerosene-like taint which is probably due to presence of volatile aromatic hydrocarbons (Shipton et al., 1970; Ogata and Miyake, 1973). The uptake of oil drops by zooplankton after an oil spill and their elimination in faecal matter has been observed by Conover (1971) and Parker (1970). Work by several French laboratories has focussed on the concentration of benzopyrene, a carcinogenic hydrocarbon found in petroleum, in various species of fish, invertebrates and algae (Mallet and Priou, 1967; Mallet et al., 1967).

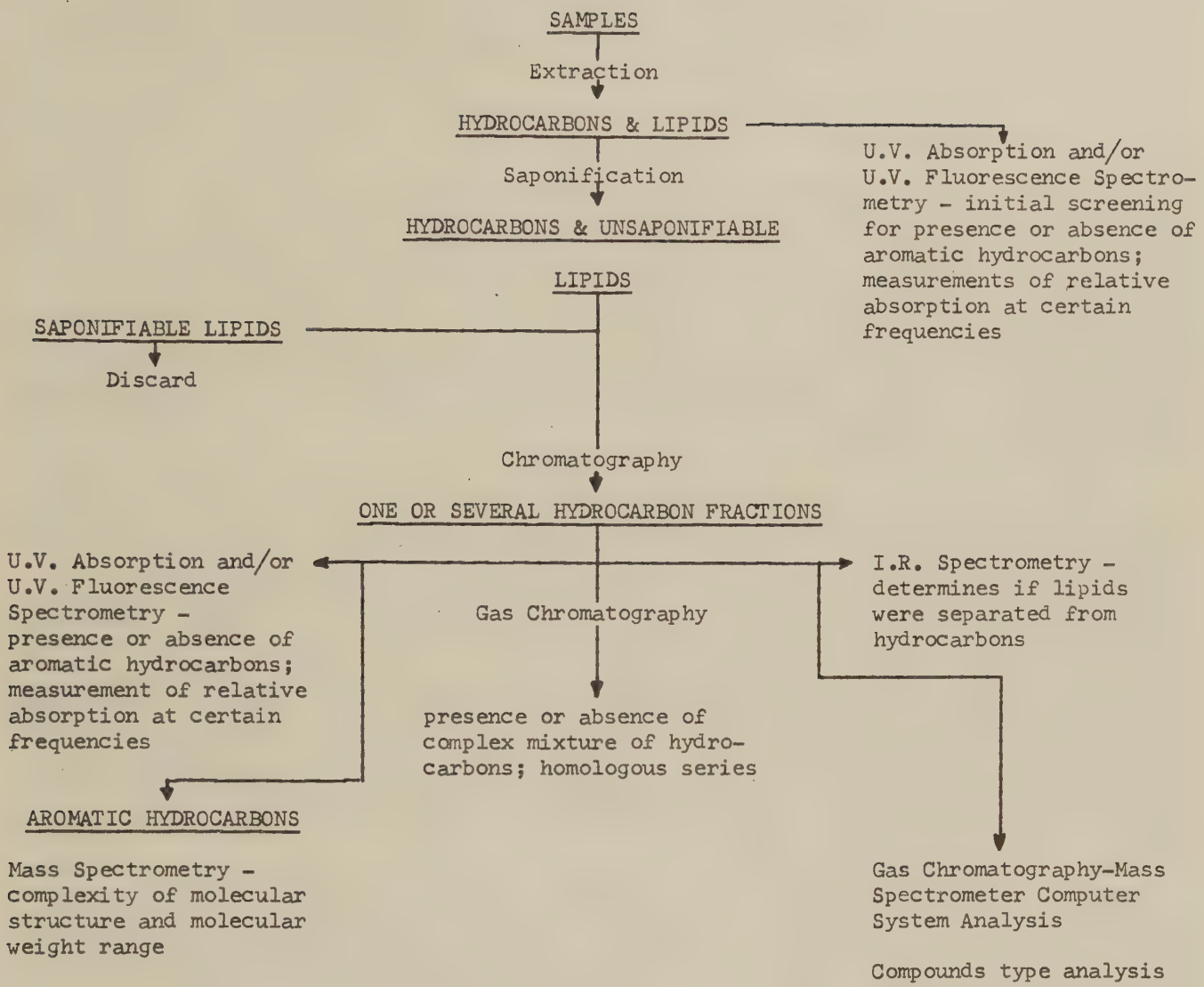
SAMPLES
Extraction
HYDROCARBONS & LIPIDS
U.V. Absorption and/or U.V. Fluorescence Spectrometry - initial screening for presence or absence of aromatic hydrocarbons; measurements of relative absorption at certain frequencies
Saponification
HYDROCARBONS & UNSAPONIFIABLE
LIPIDS
SAPONIFIABLE LIPIDS
Discard
Chromatography
ONE OR SEVERAL HYDROCARBON FRACTIONS
U.V. Absorption and/or U.V. Fluorescence Spectrometry - presence or absence of aromatic hydrocarbons; measurement of relative absorption at certain frequencies
Gas Chromatography
presence or absence of complex mixture of hydrocarbons; homologous series
I.R. Spectrometry - determines if lipids were separated from hydrocarbons
AROMATIC HYDROCARBONS
Mass Spectrometry - complexity of molecular structure and molecular weight range
Gas Chromatography-Mass Spectrometer Computer System Analysis
Compounds type analysis

Figure 1. Flow diagram for analytical techniques to detect and estimate petroleum contamination in marine organisms
(From Farrington, 1973)

The concentrations found in the tissues were highly variable but polluted coastal areas generally appear to have higher concentrations than other areas. Levels of petroleum hydrocarbons in a wide variety of marine macro-organisms are presented in the recent National Academy Report (Ocean Affairs Board, 1975). In general, the data show highest levels in animals and plants exposed to a large oil spill with lesser levels in areas of chronic oil pollution.

4.3.2 Bioaccumulation

According to data presented in the National Academy Report, the concentration of hydrocarbons in marine waters varies from approximately 3 µg/l in open water, to 20 to 50 µg/l in inshore coastal waters, and to 100-1000 µg/l in oil spill and outfall areas. There are many different methods proposed for petroleum hydrocarbon analysis and many of the apparently conflicting results concerning the concentration of hydrocarbons in the water column may be related to this controversy and also to the difficulty of extracting large volumes of water containing relatively low concentrations of hydrocarbons. The benthic animals and plants from areas of high petroleum input generally have petroleum concentrations in their tissues several orders of magnitude higher than in the surrounding water. The hydrocarbon concentration of various organisms collected in areas of high petroleum input are presented in Table I.

Thus, some of the hydrocarbons taken up from water and/or food are stored within the different tissues of marine animals. Perhaps because of their high lipid content, the liver in marine fish and the hepatopancreas of several invertebrates are sites of hydrocarbon storage (Lee, Sauerheber and Benson, 1972; Lee, Sauerheber and Dobbs, 1972; unpublished data). The gall bladder in fish is also a temporary storage site, although this organ apparently serves mainly as an avenue for discharge. Some evidence indicates that the various types of petroleum hydrocarbons have different retention times. For example, oysters exposed to oil contaminated waters accumulate aromatic hydrocarbons to a greater extent that paraffinic hydrocarbons (R.D. Anderson, 1973; Blumer, Souza and Sass, 1970). Fish given Kuwait crude in the food retained the longer chain hydrocarbons but not short chain alkanes aroun' carbon 16 and below (Hardy et al., 1974). Processes which could cause an organism not to reflect the petroleum composition in its external environment include selective uptake, selective metabolism and selective excretion.

4.3.3 Biogenic hydrocarbons

It is appropriate at this point to discuss the "natural" concentrations of hydrocarbons in supposedly uncontaminated organisms. These so-called biogenic hydrocarbons are generally long chain paraffinic hydrocarbons (Carbon chain length between C_{12} to C_{30}) with an odd carbon number predominance. The biogenic hydrocarbon concentration is 1 to 2 µg/g or less. Thus, the presence of petroleum hydrocarbons in animal tissues can be distinguished by a higher hydrocarbon content and also by a detailed analysis of the hydrocarbon fraction, since petroleum contains aromatic hydrocarbon and also the odd-even carbon number ratio is one. For example Ehrhardt (1972) reports hydrocarbon concentrations of 236 µg/g in oysters from the Houston ship channel. Oysters from nearby uncontaminated areas have approximately 2 µg/g total hydrocarbon. Zitko (1971) noted that starfish from an oil spill area had hydrocarbon concentrations between 20 and 147 µg/g while starfish from an unexposed nearly area contained 3 µg/g.

4.3.4 Hydrocarbon metabolism

Complicating factor in any study of bioaccumulation of hydrocarbons is the rate of hydrocarbon metabolism. All vertebrate and some invertebrate systems that have been examined have a so-called "detoxifying system" which facilitates elimination of lipid soluble foreign compounds from the organisms by the addition of polar groups to the hydrocarbon molecule thus increasing its water solubility. Involved are a series of enzymes which carry out hydroxylation and conjugation reactions. The enzyme systems are non-

Table I

Amount of hydrocarbon in organisms from petroleum contaminated areas
(after Anderson et al., 1974)

Organism	Probable Source	Hydrocarbon Type	Analysis	Hydrocarbon Concentration µg/g	Reference
Macro algae					
Fucus sp.	Spill - Bunker C	n-Paraffins	GC	5.8	Clark et al., 1973
Snails					
Littorina littorea	Spill	Bunker C aromatics	Fluoro	27-600	Scarratt and Zitko, 1972
Clams					
Mercenaria mercenaria	Sewage effluent	C_{16-32}	GC	16	Farrington and Quinn, 1973
Mya arenaria	Spill	Fuel Oil Number 2	GC/MS	26	Blumer et al., 1970
Oysters					
Crassostrea virginica	Chronic	Paraffins, mono and di-aromatics	GC/MS TLC	236	Ehrhardt, 1972
	Spill	Fuel Oil Number 2	GC/MS	70	Blumer, Souza and Sass, 1970
	Chronic	Polynuclear aromatics	UV	1	Cahnmann and Kuratsane, 1957
	Chronic	Total HC	GC	160	R.D. Anderson, 1973 (Galveston, Red Bluff Reef)
	Chronic	Saturates, C_{12-24}	GC	11.2	R.D. Anderson, 1973 (Galveston, Halfway Reef)
		Dimethylnaphthalenes	GC	0.6	"
		Trimethylnaphthalenes	GC	0.6	"
Mussels					
Modiolus modiolus	Spill	Bunker C aromatics	Fluoro	21-372	Scarratt and Zitko, 1972
Mytilus edulis	Spill	Bunker C aromatics	"	77-103	Zitko, 1971
	Chronic - harbour	n-Paraffins	GC	0.97	Clark and Finley, 1973
	Spill - Fuel Oil Number 2	"	GC	1.4	"
Mytilus californianus	Spill - Bunker C	"	GC	0.87	"
Scallops					
Aequipecten irradians muscle	Spill	Fuel Oil Number 2	GC	7-14	Blumer et al., 1970

Table I (continued)

Organism	Probable Source	Hydrocarbon Type	Analysis	Hydrocarbon Concentration ug/g	Reference
Barnacles					
Mitella polymerus	Spill - Bunker C	N-Paraffins	GC	11.8	Clark *et al.*, 1973
Crabs					
Cancer irroratus	Spill	Bunker C aromatics	Fluoro	7-11	Scarratt and Zitko, 1972
	Spill - Bunker	n-Paraffins	GC	2.9	Clark *et al.*, 1973
Lobster					
Homarus americanus					
gut	Spill	Bunker C aromatics	Fluoro	103-130	Scarratt and Zitko, 1972
stomach	"	"	"	15-230	"
claw muscle	"	"	"	2-3	"
abdominal muscle	"	"	"	1-4	"
Urchins					
Strongylocentrotus droebachiensis	Spill - Bunker C	Bunker C aromatics	Fluoro	17-94	Scarratt and Zitko, 1972
Mullet					
Mugil cephalus flesh	Chronic - harbour	Kerosene taint	GC/MS	860	Shipton *et al.*, 1970
Whitefish - flesh	Spill	Diesel oil	GC	29-88	Ackman and Noble, 1973
Flatfish	Chronic - coast	C_{14-20}	GC	4	Bowen, 1971

specific and act on a variety of foreign compounds. Although the biochemical changes are often referred to as detoxification reactions, in some cases the changes result in an increase in toxicity. In mammals, xenobiotic metabolism is assumed to occur primarily in the liver and kidney, although recent work has also implicated the gastrointestinal tract as a site of metabolism (Hartiala, 1973). A characteristic of many polycyclic hydrocarbons is their tendency in mammals to induce the synthesis of metabolizing enzymes, particularly the mixed-function oxidase system. This can be seen by assaying enzyme activity and also by electron microscopy of liver tissues. The latter is evidenced by an increase in smooth membranes of the endoplasmic reticulum. Since the hydrocarbon metabolism enzymes are located in the microsomes, it is assumed that the enzymes are synthesized in the new smooth membranes. This increase in enzyme activity presumably accelerates the degradation of the hydrocarbons. Recent work by Pederson et al. (1974) shows that benzpyrene hydroxylase can be induced in rainbow trout.

Degradation of aromatic and paraffinic hydrocarbons occurs in marine fish and crustaceans (Corner et al., 1973; Lee, 1975; Lee, Sauerheber and Dobbs, 1972). However, the extent of hydrocarbon metabolism in other organisms belonging to other phyla is not known at the present time. Oysters, mussels and other benthic molluscs remove hydrocarbons from the water while filtering large quantities of water, but appear to lack the ability to metabolize these compounds (Lee, Sauerheber and Benson, 1972). Preliminary work with jellyfish and ctenophores suggests that hydrocarbon metabolism is slow or absent. Such organisms tend to store larger amounts of petroleum than do organisms where metabolism is rapid. However, even where metabolism is lacking, there is evidence of excretion.

4.3.5 Relating analytical studies to bioaccumulation from water and/or food

There have been a number of laboratory studies where petroleum hydrocarbons were added to the water or food and the accumulation of hydrocarbons by an organism was determined. Stegeman and Teal (1973) exposed oysters to Fuel Oil Number 2 at 106 µg/g for 98 days and found that the highest concentration reached in the oysters was 335 µg/g. This can be compared with the 236 µg/g hydrocarbon concentration for oysters from Houston ship channel (Ehrhardt, 1972) or the 70 µg/g in oysters exposed to the Fuel Oil Number 2 spill in Buzzards Bay (Blumer, Souza and Sass, 1970). When oysters were exposed to Fuel Oil Number 2 at 11 µg/g, a level of 35 µg/g was found to be present after 28 days.

J.W. Anderson (1973), Clark and Finley (1975); Lee, Sauerheber and Benson (1972), and Lee, Sauerheber and Dobbs (1972) have carried out depuration experiments with molluscs, crustaceans and fish where animals are allowed to take up petroleum hydrocarbon from the water followed by transfer of the animals to clean sea water. J.W. Anderson (1975) allowed clams, fish and shrimp to take up Fuel Oil Number 2 for 24 hours. After 15 days of depuration, naphthalenes were still present in the clams (0.8 µg/g) but were below detectable levels in fish and shrimp. Clark and Finley (1974) found a concentration of 8 µg/g paraffin hydrocarbons in the mussel, Mytilus edulis, after 2 days exposure to Fuel Oil Number 2. After two weeks of depuration the paraffin concentration was 0.4 µg/g. When Mytilus edulis was exposed to mineral oil as well as to various radio-labeled hydrocarbons, there was significant accumulation of hydrocarbon but after two weeks of depuration approximately 90% of the hydrocarbon was lost from the tissues (Lee, Sauerheber and Benson, 1972).

Based on laboratory and field studies, it appears that bioaccumulation of petroleum hydrocarbons by marine organisms does occur. However, these studies are complicated by hydrocarbon metabolism and excretion. In an oil spill area or in areas of chronic oil pollution, the tissues of certain marine organisms may reflect the total concentration of petroleum in the water and also the concentration of the different hydrocarbons in the water. However, where extensive depuration can occur because of clean sea water being brought in by tidal action, etc., it is expected that the hydrocarbon pattern in the animals will be modified from the pattern in the water.

4.4 Bioaccumulation of Petroleum Hydrocarbons - Selection of Organisms

Laboratory and field studies indicate that petroleum hydrocarbons are accumulated by marine organisms. The extent of metabolism and excretion of the hydrocarbons complicates the choice of marine organisms for monitoring purposes. In an oil spill area or in areas of chronic oil pollution, the tissues of certain marine organisms may reflect the total concentration of petroleum and the relative amounts of different hydrocarbons in the water, i.e. the hydrocarbon pattern. However, as discussed earlier, extensive depuration can occur if clean sea water replaces the contaminated water and this will result in a different hydrocarbon pattern.

Additional uses of studies on bioaccumulation of petroleum hydrocarbons by marine organisms include (1) the ability to monitor carcinogenic hydrocarbon input which, because they are a small fraction of petroleum and because of their low concentration, are difficult to analyse in the water; (2) allows attention to be focussed on biologically active hydrocarbons, i.e. hydrocarbons taken up by living organisms and (3) provides a reliable method of monitoring a petroleum clean up of an area by observing if the animals' concentration of hydrocarbons decreases.

Based on present evidence bivalves, specifically clams, oysters and mussels, appear to be a group of animals which are suitable for monitoring petroleum hydrocarbons. Their advantages are (1) their slow hydrocarbon metabolism (Carlson, 1972; Lee, Sauerheber and Benson, 1972); (2) ability to bioaccumulate hydrocarbons under both field and laboratory conditions (R.D. Anderson, 1973; Ocean Affairs Board, 1975; Blumer, Souza and Sass, 1970; Clark and Finley, 1973; Ehrhardt, 1972; Farrington and Quinn, 1973; Fossato and Siviero, 1974; Stegeman and Teal, 1973); (3) extensive knowledge of their biology and physiology (Harger, 1972; Jørgensen, 1966; Nixon et al., 1971; Thompson and Bayne, 1972); (4) the fact that they can be maintained under laboratory conditions for long periods (Jørgensen, 1966; Thompson and Bayne, 1972); (5) their world-wide distribution including oil polluted water (Clark et al., 1973; Ehrhardt, 1972; Farrington and Quinn, 1973; Fossato and Siviero, 1974) and (6) the large size of animals and ease of tissue dissection (Lee, Sauerheber and Benson, 1972).

The suggestion of bivalve use is not meant to exclude from consideration other groups of marine organisms which may in the future be shown to bioaccumulate petroleum hydrocarbons (Ocean Affairs Board, 1975).

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5. MONITORING OF CHLORINATED HYDROCARBONS

by

R.F. Addison

5.1 Introduction

This chapter deals mainly with three groups of organochlorine compounds: the DDT group of insecticides, comprising p,p'-DDT and its metabolites, principally p,p'-DDD and p,p'-DDE, the polychlorinated biphenyls (PCBs) and the cyclodiene insecticides, mainly aldrin and dieldrin. For the purposes of this review, these materials will be described collectively as "chlorinated hydrocarbons". All three groups share the common properties of (a) relative chemical stability, which accounts for their persistence, (b) appreciable volatility at normal environmental temperatures, which accounts for their transport in the vapour phase and (c) relative water insolubility, lipid solubility and high lipid/water partition coefficients which account for their tendency to be stored in an organism's depot fat. There are, however, variations between and within groups, in the extent to which these properties are represented. Since the environmental distribution and transport of these materials appears to be governed by their physical properties, other materials with generally similar properties (such as brominated biphenyls, chlorinated naphthalenes and chlorinated paraffins) might be expected to behave similarly, although there may be as yet only limited information about their environmental distribution.

5.2 Inputs

5.2.1 Production

The DDT and cyclodiene groups are used as insecticides, and as such released deliberately into the environment; the PCBs, on the other hand, have various industrial uses, and are released into the environment more or less unintentionally - except where they are added to insecticide formulations to slow the release of insecticide.

Figures for world production and input to the environment have been estimated for the DDT group and for PCBs. Total integrated world production of DDT has been assumed to be 2×10^6 metric tons, with an annual production (or input to the environment) of 10^5 metric tons (SCEP, 1970). The latter estimate is probably on the low side, as it is less than U.S. production in 1965 or 1967 (American Chemical Society, 1969). PCB production in the U.S. peaked in 1970 (Hutzinger et al., 1974) at about 3.3×10^4 metric tons, but thereafter fell off rapidly. It has been estimated that U.S. production of PCBs may account for about one half world production (Harvey et al., 1973). Residues in marine biota account for only a small fraction of the world's production of DDT, but may represent a much larger fraction of the world's production of PCBs (SCEP, 1970; Harvey et al., 1973).

5.2.2 Transport

Both DDT and PCBs are transported primarily by atmospheric transport rather than by surface run-off (SCEP, 1970; Harvey et al., 1973); atmospheric residues apparently exist largely in the free vapour, rather than adsorbed to particulate material (Bidleman and Onley, 1974).

5.3 Analytical Procedures

The general approaches and detailed procedures used in the analysis of chlorinated hydrocarbons are described elsewhere (U.S. Department of Health, 1969; FAO, 1975). It should be recognized that any method may require small modifications, e.g. to the composition of chromatographic eluants, or to GLC column composition, to achieve the analyses

required. Where such modifications are made, appropriate blank or calibration samples should be run.

5.4 Distribution

5.4.1 Uptake

Aquatic organisms can accumulate chlorinated hydrocarbons by three routes: (a) adsorption to external surfaces; (b) absorption through external tissues such as gill, and (c) ingestion of contaminated food. Adsorption seems to be the main uptake route in small planktonic organisms (Kerr and Vass, 1973) and whether adsorbed material actually penetrates within phytoplanktonic cells is not altogether clear (Cox, 1972). In invertebrates, both ingestion and adsorption to external surfaces contribute to uptake; ingestion appears to predominate with increasing size (Kerr and Vass, 1973). In fish, ingestion appears to be the main uptake route in practice (Macek and Korn, 1970; Norstrom et al., in press) except immediately after sudden increases in water concentrations of chlorinated hydrocarbon. In this situation absorption through the gill may be significant during the intervening period before components in the fish's diet accumulate increased residue concentrations (Hamelink et al., 1971). In mammals and birds, ingestion would appear to be the only route of uptake.

Chlorinated hydrocarbons are accumulated with varying degrees of efficiency depending on their structure. In fish (the organisms most extensively studied) the efficiency of accumulation by either ingestion or gill absorption generally increases in the sequence lindane (a hexachlorocyclohexane) < dieldrin < DDT < PCBs (Gakstatter and Weiss, 1967; Grzenda et al., 1970; Grzenda et al., 1972; Lieb et al., 1974).

5.4.2 Elimination

The elimination of chlorinated hydrocarbons by aquatic organisms is generally much slower than their uptake. Thus, although concentrations of p,p'-DDT accumulated from water by phytoplankters may reach equilibrium within a few seconds, clearance requires several days to reach completion (Södergren, 1968). Uptake and clearance of chlorinated hydrocarbons by fish takes correspondingly longer; many experimental studies have been terminated before clearance was completed, but assuming an exponential clearance process the data suggest that the half-life of chlorinated hydrocarbons in fish would be of the order of several weeks (Grzenda et al., 1970; Hattula and Karlog, 1973). The elimination of chlorinated hydrocarbons depends to some extent on structure: studies on fish have shown that the efficiency of elimination increases in the sequence PCB < DDT < dieldrin < lindane (Grzenda et al., 1970; Hattula and Karlog, 1973).

To summarize the experimental results of studies on the uptake and elimination of chlorinated hydrocarbons by aquatic organisms: routes of uptake include adsorption, absorption and ingestion, with ingestion predominating in larger organisms; the rate of uptake is generally much greater than that of elimination, and the tendency for chlorinated hydrocarbons to be retained increases in the sequence lindane < dieldrin (and other cyclodienes?) < DDT < PCBs.

5.4.3 Factors affecting storage

One consequence of the fact that rates of residue uptake exceed those of elimination is that the chlorinated hydrocarbon burden of an organism increases with time. This may often be reflected in an increase in residue concentration with the age of the organism provided the source of chlorinated hydrocarbons remains fairly constant, and given an appropriate growth curve. Such relationships have been established for both the DDT group and PCBs in freshwater and marine fish (Bache et al., 1972; Youngs et al., 1972; Jensen et al., 1972); for the DDT group and PCBs in seals (Addison et al., 1973) and for dieldrin in sea birds (Robinson et al., 1967). The form of the increase may vary: both exponential (Bache et al., 1972) and linear (Addison and Smith, 1974), relationships have been described but these differences probably reflect the various assumptions made in comparing the analytical

data (Addison et al., 1973). However, the data demonstrate the general point that clearly the organism's age is one factor which may affect its residue content.

Chlorinated hydrocarbon residues are generally stored in an organism's depot fat, and their concentration on whole-body basis may depend on how the organism "handles" its fat reserves. Two extreme cases can be envisaged: in one case, the organism may handle residues completely independently of the way in which it handles the fat in which they are stored; in the other, handling of residues and fat may be unselective. An example of the former situation is found in Atlantic herring, where, despite wide seasonal variations in fat content of the fish, residue concentrations (DDT and PCB) expressed on a whole-body basis stay reasonably constant (Addison et al., 1972; Hutzinger et al., 1974). They vary, of course, inversely to the fat content of the fish. This is not necessarily a general feature of the chlorinated hydrocarbon-fat relationship in fish: both positive and negative correlations between residue concentrations and fat contents, both expressed on a whole-body basis, have been observed (Earnest and Benville, 1971).

An example of the non-selective handling of residues and fat is found in the female seal. Residue concentrations in blubber increase with age in male, but not in female seals (Addison and Smith, 1974). The difference is apparently due to the fact that the female sheds a large fraction of her residue burden during mobilization of depot fat into milk, during lactation (Anas and Wilson, 1970; Addison and Brodie, unpublished data). This, incidentally, illustrates the point that residue concentration in an organism may also depend on its sex. In general, then, variations in the fat content of an organism (whether arising through starvation or normal metabolic processes) should be recognized as another factor which may influence an organism's residue burden.

5.4.4 Metabolism

Chlorinated hydrocarbons may be metabolized by marine organisms. From the data available, it seems that in general the degradative pathways are similar to those found in terrestrial animals (for reviews of established routes see O'Brien, 1967; Menzie, 1969; Fukuto and Sims, 1971), but the processes go more slowly, at least in poikilotherms. The slower rates may be attributed partly to the lower temperatures at which such animals operate (Zinck and Addison, 1975). The conversion of p,p'-DDT to p,p'-DDD and to p,p'DDE by marine micro-organisms (Patil et al., 1972), phytoplankton (Bowes, 1972), lobsters (Guarino et al., 1974) and fish (Addison and Zinck, in press) has been demonstrated experimentally. Distributional evidence suggests that marine mammals can also carry out the p,p'-DDT to p,p'-DDE conversion (Addison et al., 1973). Experimental studies have also shown that fish can convert p,p'-DDD to p,p-DDMU (Addison and Zinck, in press) and p,p'-DDT to p,p'-DDMU and to p,p'-DDA (Pritchard et al., 1973) but since these conversions occur so slowly, and since the products are not routinely analysed in monitoring operations, they can be ignored for the purposes of this review. Phenolic metabolites of p,p'-DDE have been detected in faeces from seal and guillemot (Jansson et al., 1975). The conversion of aldrin to dieldrin by micro-organisms (Patil et al., 1972), lobsters (Carlson, 1974) and fish (Ludke et al., 1972) has also been demonstrated. Only limited information exists about the capacity of marine organisms to degrade PCBs: several marine organisms can hydroxylate biphenyl (Willis and Addison, 1974) and we might conclude that this suggests that clorobiphenyls might also be hydroxylated, although considerably more slowly. Seals and guillemots can in fact hydroxylate some chlorobiphenyls (Jansson et al., 1975).

5.4.5 Interactions among group members

The general conclusion suggested by the literature is that while chlorinated hydrocarbons may be metabolized to some extent by marine organisms, the processes occur so slowly that under normal circumstances metabolism is not a factor which would seriously affect an organism's residue burden. However, there is one rider to be attached to that general statement: that is the fact that certain interactions of chlorinated hydrocarbons may occur. Thus, when fish were fed with p,p'-DDT, dieldrin and methoxychlor, the presence of either

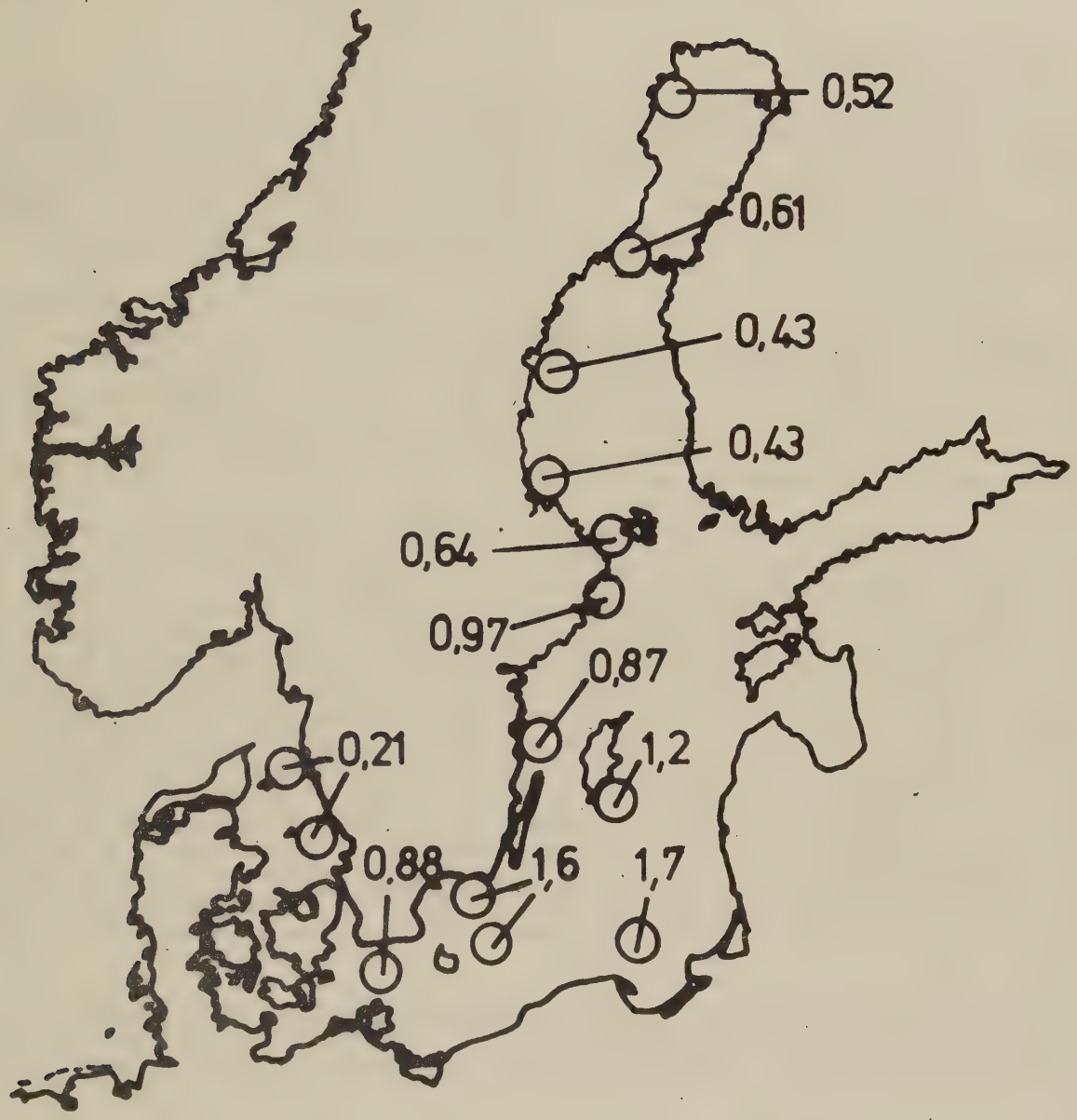

Figure 1. Variation in total DDT levels (ppm wet weight) in herring from various locations in the Baltic area. (Calculated and redrawn from Jensen et al., 1972)

p,p'-DDT or methoxychlor stimulated elimination of dieldrin, and both p,p'-DDT and dieldrin feeding reduced methoxychlor storage (Mayer et al., 1970). It is difficult to assess the significance of these results to data from monitoring operations, but the analyst should be alert to the fact that such complex interactions can occur, and should interpret his data accordingly.

5.5 Species Selection

Since the accumulation of chlorinated hydrocarbons by marine organisms is governed by various physiological and environmental factors, the use of biological accumulators in environmental monitoring seems most suitable for providing comparative, rather than absolute, information; i.e. they would be used to detect differences in environmental pollutant levels, rather than to reflect absolute levels. Two sorts of comparison seem valuable: those made on a spatial basis (where one marine area is compared with another) and those made on a temporal basis (where variations over a period of time in any one area are assessed).

Some general criteria for selection of accumulator organisms have been listed elsewhere in this manual and, as noted above, the relationships between the organism's residue burden and its age and fat content should also be considered. Extra criteria for selection of organisms on which to base spatial comparisons include (a) that the organism should be available, and should occupy the same ecological niche, throughout the areas to be compared; (b) that the organism should not migrate between the areas to be compared or, at least that it should not accumulate appreciable portions of its residue burden during such migrations.

Sessile organisms are obvious candidates for such spatial comparison, but they apparently have been used in only two such programmes so far (Butler, 1973; ICES, 1974). In one of these (Butler, 1973) considerable spatial variation in residue levels was detected among molluscs of the same species sampled at the same time, and in many cases it was possible to attribute such variations to the effects of local agricultural use of certain insecticides. Because of the choice of sampling points, it is difficult to assess the minimum distance which would separate areas recognizably distinct in terms of their chlorinated hydrocarbon contamination, but a resolution of 100 km or even less seems plausible. Fish have also been used as accumulator organisms for such comparisons, and analyses of cod have permitted comparison of pesticide inputs to adjacent Norwegian fjords (Sternsen and Kvalvag, 1972). Chlorinated hydrocarbon residues in commercial catches of Baltic herring reflected differences in the extent of contamination of their environment; resolution of areas which had distinct differences in environmental contamination was of the order of 100 km (Jensen et al., 1972) (Fig. 1). This conclusion is particularly interesting as no deliberate effort was made to eliminate the effects of variables such as age, fat content or migratory behaviour during sampling. The relevant data were recorded, however, for inclusion in assessment of analytical data.

Regional differences have been detected in chlorinated hydrocarbons in mussel and shrimp collected from the North Sea coasts (ICES, 1974). In that study, herring proved to be a rather insensitive indicator of regional differences, presumably because of their migratory habits.

DDT concentrations in the crab Emerita analoga taken from various points along the Californian coast showed reflected local variations in inputs over relatively short distances (<100 km: Burnett, 1971).

Temporal variations in environmental contamination can also be, and have been, detected through analyses of accumulator organisms. In addition to the general criteria above, organisms should be chosen so that their life span is of the same order as, or less than, the time intervals being compared.

Analyses of small marine plankton showed dramatic fluctuations in PCB levels, which correlated well with previous rainfall patterns; these results suggested that such organisms

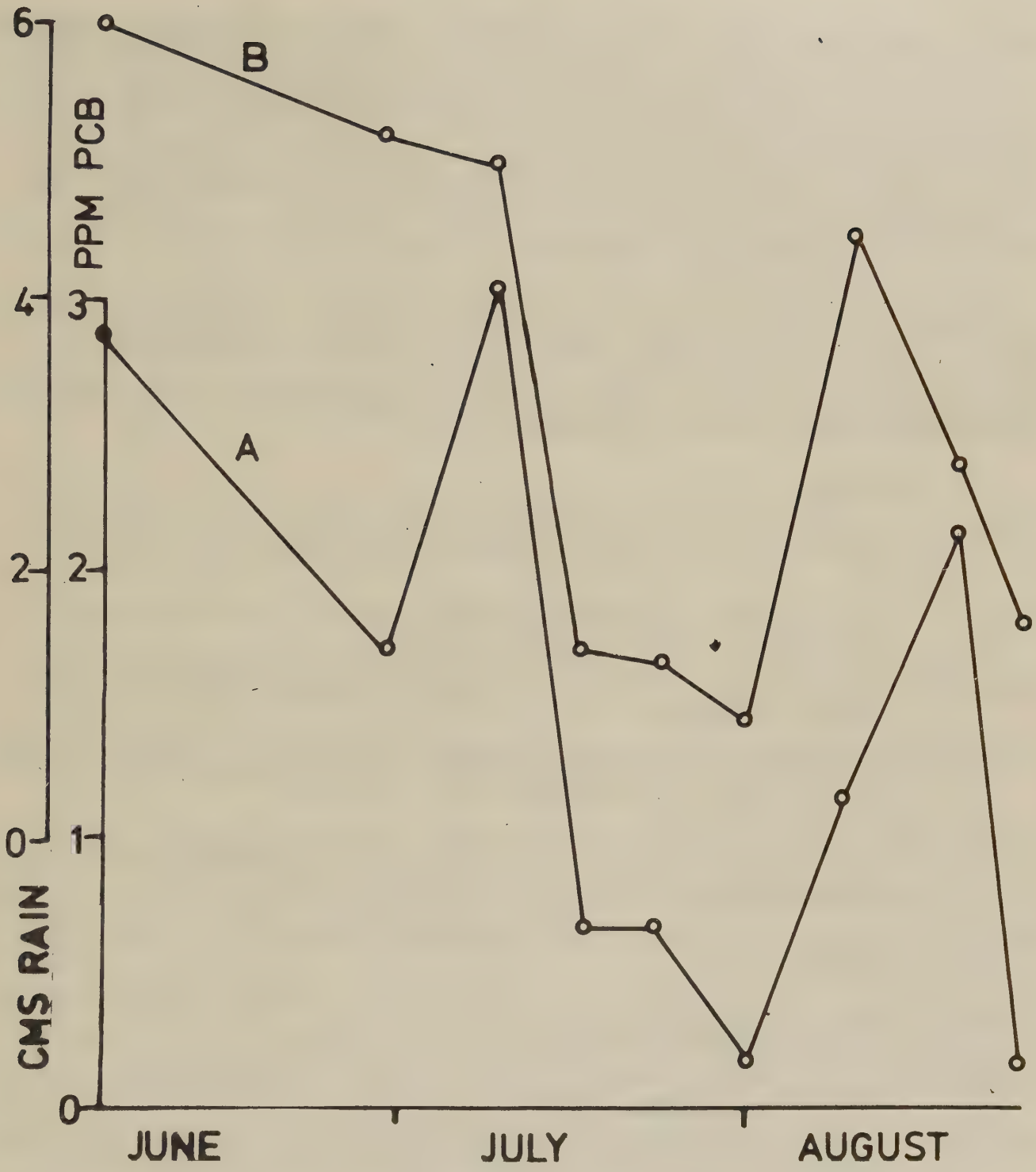

Figure 2. Comparison of PCB levels (ppm wet weight) in No. 20 mesh plankton collected from the Gulf of St. Lawrence at intervals over a 3-month period (curve A), with cumulative rainfall (cm) in the same area during the 10-20 days before sampling (curve B). (Calculated and drawn from Ware and Addison, 1973)

could be used to detect short-term fluctuations (of the order of 10 days or so) in input of PCBs to the marine environment (Ware and Addison, 1973) (Fig. 2). Rather longer term changes - of the order of years - can be, and have been, detected through use of fish and shellfish as accumulator organisms. Analyses of myctophids from the Californian coast showed gradually increasing concentrations of DDT from 1949 until the early 1970s, which presumably paralleled increases in marine environmental contamination (McGregor, 1974). The shellfish monitoring programmes (Butler, 1973) also illustrated changes in environmental contamination by organochlorine pesticides; following a period of generally level residue concentrations from 1965 to 1969/70, residue concentrations in these animals began to show a consistent decline from about 1970 onwards.

The use of accumulator organisms to detect either spatial or temporal differences in the extent of chlorinated hydrocarbon contamination of the marine environment thus seems well established. It seems probable that future programmes will be directed towards detecting relatively large scale spatial differences, say of the order of 100-1000 km, or relatively long-term temporal changes (of the order of a year or more), and so suitable organisms could be selected from commercially available catches of fish or shellfish, provided that the general criteria listed above are met.

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6. MISCELLANEOUS SUBSTANCES

by

S. Jensen

6.1 Introduction

This group of substances includes two categories, those known to be present in environmental samples and those which are known to be released into the environment but which have not yet been detected in environmental samples.

6.2 Halogen Compounds

6.2.1 Chlorinated aromatics

Polychlorinated terphenyls (PCT) and polychlorinated naphthalenes (PCN) have been used as supplements to PCB and have also been proposed as substitutes for PCB in countries where PCB has been banned by law. (Zitko and Choi, 1971).

Extraction and subsequent clean-up of PCT and PCN can be accomplished using the same methods as those adopted for PCB. For PCN the same GC system can be used, although analysis of PCT is more difficult because of its very low vapour pressure. This problem with PCT can be overcome by using extremely short columns (about 20 cm). When PCT is not pretreated no distinct peaks will appear under normal organochlorine pesticide analysis conditions. Pretreatment of PCT can be achieved either by dehalogenation and subsequent detection with flame ionization detection or PCT can be totally chlorinated. Either of these methods will give rise to distinct peaks.

6.2.2 Chlorinated aliphatics

These substances, many of which are used as solvents, may be formed as byproducts in industrial processes and have been found to accumulate to some extent in marine organisms. (Jensen et al., 1972). Thus, chloroform, carbon tetrachloride and perchloroethylene are found in many organisms in the sea. (Pearson and McConnel, 1975). Furthermore, a great variety of aliphatic chlorinated hydrocarbons from dichloroethane up to pentachlorobutenes (the so-called EDC tar components) are formed as by-products in the production of vinyl chloride. This residue has been dumped at sea in drums and into the surface waters and has subsequently been detected in fish. (Jensen et al., 1975). Because of their relatively high vapour pressure these substances are lost in the normal procedure for residue analysis. There are several methods by which environmental samples can be analysed for aliphatic hydrocarbons. In one of these, the sample to be analysed is homogenized and placed in steam-distillation apparatus together with a few millilitres of hexane and an anti-foaming agent. The substances will pass over into the collecting vessel together with a reasonable volume of water. After treatment of the hexane extract with concentrated sulphuric acid, analysis can be carried out using GLC with electron capture detection. The liquid phase loading on the column packing normally needs to be at least 10 ml and a temperature of around 110°C is necessary in order to achieve reasonable retention times.

6.2.3 Chlorinated dibenzo-p-dioxins

These substances are present as impurities in a range of chlorinated organic compounds, for example in certain low grade forms of the herbicide 2,4,5-trichlorophenoxy acids (Langer et al., 1973) or in fungicides such as pentachlorophenol. (Jensen and Renberg, 1973; Plimmer, 1973). They are persistent and lipophilic and have been found in freshwater fish in some areas of the world (Baughman and Meselson, 1973). They can be extracted from biological samples using the same procedure as that adopted for PCB and DDT. Retention times

on the gas chromatograms are rather long when the normal conditions are used. The most prominent member of the class of compounds, 2,3,7,8-tetrachloro-dibenzo-p-dioxin, is extremely toxic. (Miller et al., 1973). Consequently, only very low concentrations are likely to be found in marine organisms and the highest standards of analytical sophistication are required if residues of the substances are to be detected.

6.2.4 Pentachlorophenol and lower homologues

These compounds are used as such, e.g. as fungicides, but they are also formed as metabolites of chlorinated benzenes and other compounds, and have been detected in environmental samples. (Renberg, 1973). If special care is not taken, they are likely to be lost in the extraction stage of analysis since, due to their acidic properties, it is necessary to acidify the sample before extraction. They must also be either methylated or acetylated before injection into a gas chromatograph. Thus, their presence can easily be recognized if the same extract is run on a gas chromatograph before and after derivatization.

6.2.5 Unidentified halogen compounds

Analysis of lipid fractions extracted from marine organisms has revealed the presence of high concentrations of organically bound chlorine (20-650 mg/kg) bromine (2-60 mg/kg) and iodine (0.5-60 mg/kg). Up to 50 mg/kg of chlorine was found in herring fat from fish caught both off the Swedish west coast and in the Baltic. (Lunde and Steinness, 1975). The amount of chlorine which could be accounted for as PCB or DDT was at the most 20 mg/kg. There is usually a large discrepancy between the total chlorine content of a sample and the amount which can be accounted for by the presence of known organohalogens. However, there is evidence that compounds of this type can be formed in the marine environment and there is a possibility that the major part of these organohalogen compounds is of biogenic origin. Provided the analyst has ready access to neutron activation facilities, total organically bound halogen analysis is not expensive and may provide useful data relevant to the detection of sources of contamination. More detailed information can be obtained if the extract is separated into fractions by means of steam distillation, or if an aliquot is treated with either a base or with concentrated sulphuric acid.

6.3 Halogen-free Compounds

The most important group of compounds in this class are the plasticizers such as the esters of phthalic acid. Thus, high levels of octyl and butyl phthalates have been reported in marine organisms. (Stalling et al., 1973). However, at least some of these figures are of doubtful validity due to inaccurate analytical procedures, since almost all solvents used in residue analysis contain phthalates. Thus, use of an incompletely purified solvent stored in plastic bottles will give values which are too high. Although the phthalates do not contain halogens they do have a response on the electron capture detector comparable with the halogenated hydrocarbons. However, being rather polar, they can be isolated quite easily in the clean-up and separation stages of conventional residue analysis.

6.4 Laboratory Experiments

In the introduction to this manual, a distinction is made between a pollutant and a contaminant. The main difference lies in the relative level in the environment or organism and whether or not that level has an effect on the well being of the organism. It is, therefore, important that realistic laboratory tests be conducted for all substances which are found to accumulate. Thus, the accumulation test should, as far as possible, simulate the levels, routes and conditions which would apply in the sea. Only then will it be possible to state whether environmental levels have any long-term effects.

For some lipophilic substances no accumulation plateau has been found indicating that no steady state was reached during the experimental period. In these cases no overall concentration factor can be defined although it is possible to define an accumulation rate.

It should be realized that a major shortcoming in many accumulation studies is that the test organism is analyzed whole. This can lead to the total loss or at least obscuring of much valuable information and, as a general rule, each major organ should be analysed separately so as to allow the identification of the main storage organ and potential target site.

6.5 Selection of Substances to be Investigated

Before a monitoring programme is started, a pilot study should be conducted to establish whether the contaminant of interest is likely to be present. This will usually take the form of a predictive exercise involving an assessment of contaminant input data followed by a baseline survey. In programmes which include organohalogen compounds, neutron activation analysis for total organically bound halogens might yield valuable information but cost-effectiveness will be greatly influenced by the ready availability of neutron activation facilities.

At present the number of substances considered to be accumulated by marine organisms belong to only a few chemical classes. Thus, organic substances which contain halogens are usually considered to be stable and lipophilic and as such likely to be accumulated. However, some non-halogenated substances also have these properties, e.g. if they have large conjugated unsaturated systems, and some of these may also accumulate in marine organisms.

Some optical brighteners have very large chromophoric systems and are sometimes lipophilic and thus are potentially bioaccumulatable. One such substance containing sulphur, oxygen and nitrogen has been shown to be very stable to biological attack. It was accumulated by fish but also rapidly released through excretion (Jensen and Pettersson, 1971).

As a general rule molecules containing acidic oxygen, or basic nitrogen, are too polar and easily metabolized to be accumulated to any great extent by organisms.

6.6 General Comments

Estimation of total halogen content can be seen as a way of performing a relatively unbiassed search for unknown peaks. Even routine residue analyses can provide some unbiassed information if all unknown peaks of the GLC chromatogram are investigated separately. This is especially possible if the purified extracts are injected into gas chromatographs fitted with detectors sensitive to different classes of substances.

Since normal residue analysis concentrates on substances which are more lipophilic than natural fats and which have reasonable vapour pressures, many substances which do not have these properties may not be detected. It is therefore important that other methods be adopted in the future. Gel permeation shows promise and can be a useful tool in which substances are separated according to differences in size and aromaticity rather than by differences in polarity. High pressure liquid chromatography might be useful in the analysis of any non-volatile substances which may be present in the sample.

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7. A MATRIX TABLE

7.1 Introduction

From the foregoing chapters it will be apparent that certain organisms have particular abilities to accumulate contaminants. The same organism does not necessarily accumulate all contaminants to the same extent. Consequently, it is usually necessary to select a number of species according to the contaminant of interest.

In addition to the control aspect of monitoring, it is usually desired, in national, regional and global monitoring programmes, that the results obtained should be capable of revealing the presence or absence of differences in levels of contamination either by area or with time. Since the uptake of a contaminant by an organism is usually affected by certain of the biological characteristics of the organism in question, it is essential that precautions be taken to avoid the effect of such variables. Thus, if it is known that the age of an organism influences its uptake and retention of the contaminant of interest, it is essential for monitoring purposes that organisms of the same age be collected. Similarly, it may be necessary to ensure that all animals collected in an intertidal zone are collected from approximately the same relative position on a shore line relative to tidal height.

7.2 Description of the Matrix Table and its Limitations

As a quick reference guide as to which organism or type of organism might be suitable for monitoring a particular contaminant or group of contaminants, the Matrix Table (Table I) should be used. However, it should be emphasized that the table should not be used alone as a basis for designing a monitoring programme. Full reference should be made to the appropriate preceding chapters in order to ensure, for example, that the organism selected is suitable for revealing spatial differences in contaminant distribution.

The table has, for the sake of clarity, been left as simple as possible and in general merely indicates whether or not a particular group of organisms is or is not suitable for monitoring a group of pollutants. However, where positive information is available on the need to select an organism by sex, age, condition, etc., appropriate notes have been included. These notes are important and problems are likely to be encountered if they are ignored. However, the absence of notes should not be taken to mean no precautions are necessary; as mentioned earlier, further relevant comments are likely to be found in the appropriate chapters which should be read in conjunction with the table. Absence of any entry at all does not necessarily mean the organism is not suitable merely that no information is available as to its suitability.

Table I

	Organochlorine Pesticides and PCBs	Petroleum Hydrocarbons	Metals/Metalloids		Radio-nuclides	
			Cd Cr Pb Zn Cu	As and Hg	Neutron Activation Products	Neutron Fission Products
Plankton						
Phytoplankton	+ Sampling problems and short-term variations		o	o		
Zooplankton			o	o		
Macrophytes						
Green					+	+
Brown			o		+	+
Red			+		+	+
Polychaetes			+			
Molluscs (benthic)						
Bivalves	+	+	+	o	+	+
Gastropods			+	o		
Crustaceans (benthic)	+	o				
Fish						
Pelagic, muscle	+ Age, sex, condition important	o	o	+ Sex, age important	o	+ Caesium only
Pelagic, liver	o	o			+	o
Demersal, muscle		o	o	+ Sex, age important	o	+ Caesium only
Demersal, liver	+ Age, sex condition important	o			+	o
Marine Mammals						
Blubber	+ Age, sex condition important					

Key: + Useful organism for monitoring purposes
o Not a useful organism for monitoring purposes

No symbol merely means information on suitability is lacking or inadequate to give an assessment

8. PILOT STUDY ON MONITORING BIOACCUMULATION IN MARINE ORGANISMS

by

D.J. Reish

8.1 Introduction

The proposed pilot project can be divided into three phases: (1) a preparational phase, (2) a screening phase, and (3) an operational phase. A period of three years is necessary to carry out this study with the first two phases requiring one year and phase three requiring two years. Since many laboratories from many countries are likely to be involved in the pilot study, it is imperative to have good communication. Periodic meetings should be held for the purpose of exchange of data, evaluation of data, and general appraisal of the programme.

The proposals which follow relate to an idealistic pilot project designed to obtain the maximum of meaningful data. It is recognized that, as an ideal, it will be difficult to carry out in entirety in practice because of the limitations posed, e.g. by personnel, time and funding. It is imperative that critical evaluation of all phases of the project be made on a continuing basis, because of these limitations which will be encountered in the practical situation.

8.2 Preparational Phase

The purpose of the preparational phase is to summarize existing knowledge, assemble research capabilities, select the species, contaminants and stations to be sampled, and to coordinate the activities of the participating laboratories.

8.2.1 Literature review

(i) Previous monitoring programmes: Experience gained in the course of previous or continuing international and large-scale national programmes such as that conducted in the North Sea by ICES (1974) and the United States National Pesticide Monitoring Program (Butler, 1973) should be utilized in the detailed planning of any programme.

(ii) Pilot study area: All existing knowledge on the residue levels of contaminants in marine organisms from the pilot study area should be summarized and stored in a data bank. Insofar as possible, all data should be converted to common units to permit easier evaluation of previous knowledge.

(iii) World wide studies: References from regions outside the pilot study area should be summarized and stored in the data bank if they represent an important contribution or if they include data on an important organism found within the pilot study area.

(iv) Data Bank: All literature review data should be stored in the data bank by species, contaminant, and geographical locality.

8.2.2 Survey of research capabilities

An outline of the proposed pilot study should be circulated to those laboratories in the geographical area to determine their willingness to participate in the project.

(i) Personnel: A geographical list of interested personnel should be assembled which includes the expertise of each individual. The personnel list should also include technical assistants, including their capabilities and geographical locality.

(ii) Laboratories: A list of interested laboratories should be made. The research capabilities of each laboratory should be summarized according to personnel, equipment and space.

(iii) Ships: A list of all research boats and ships should be compiled. The capabilities of each ship should be listed according to size, geographical locality, ship board scientific equipment, costs and time available for the pilot study.

(iv) Training and Education: After assembling the research capabilities of the personnel, a thorough evaluation of the entire personnel roster should be made to determine whether or not gaps exist with regard to professional and technical assistants. It is logical to assume that there will be gaps in some scientific areas. Practical training programmes should be initiated along the lines of those conducted by FAO and SIDA on aquatic pollution. Such training programmes will be most effective if they concentrate on training the people who will carry out the actual work.

8.2.3 Selection of species for monitoring

(i) Criteria for Selection: The primary criteria for selection of a marine organism for monitoring should include, but not be limited to, the following: those species previously known to have high concentration factors for the contaminants to be monitored, commercial importance, either directly or indirectly, availability, wide distribution, represent various ecological niches, and represent various positions on the food chain. The species selected need not necessarily be known to satisfy all of these criteria as some may be inferred by interspecies comparison. The organisms chosen must be available in sufficient number throughout the pilot area and be readily available throughout the year. It would be desirable to select organisms with broad geographical and ecological distribution which would permit comparisons with other studies. Also, whenever possible, organisms should be chosen which have successfully been used in previous monitoring studies; such data would be helpful in evaluating the results obtained from monitoring studies.

(ii) Assemble Known Distributional Data of Monitoring Species: The geographical distributional data should be gathered for each species to be monitored. These data can be of a more-or-less general nature for regions outside the area to be monitored. However, within the area to be monitored, detailed distributional data should be gathered and plotted on maps. Field surveys should be conducted during the preparatory phase to fill gaps in geographical distributional data, especially in areas which are of particular importance in monitoring.

(iii) Assemble Existing Knowledge on the Biology of Selected Species: Details from previous studies dealing with the biology of the selected species should be assembled. Such studies should include, but not be limited to, ecological, reproductive, developmental, physiological, biochemical and body-burden studies. It would be desirable to have a separate file for each species, including a reprint of each study. The value of such a compilation is obvious; a researcher would have all data on the species available at hand and this would assist immeasurably in evaluation of results.

8.2.4 Selection of contaminants to be monitored

It is difficult at this time to make a general statement as to which substances should be monitored. A particular substance may be extremely important at one locality but not at another. Furthermore, a substance important today may not be important tomorrow and vice-versa. In other words, a monitoring programme should have sufficient flexibility to allow changes as the need arises at a specific locality. Selection of specific contaminants to be monitored should be made after an inventory has been made of the sources and types of wastes entering the system to be studied.

8.2.5 Summarize the sources and types of contaminants entering the system

The selection of the type of contaminant to be monitored and the summarization of the sources and types of contaminants entering the system should be made concurrently. It is impossible to consider one topic without consideration of the other. It would be extremely valuable, but probably virtually impossible, to know how much of what type of contaminant is entering the system at each locality. Difficult as it may be, a serious attempt should be made to assemble these data, since it would aid immeasurably in determining which contaminants to monitor and where. This summarization of sources and types of contaminants should be a continuous process throughout the course of the monitoring programme.

8.2.6 Intercalibration

Standardization of techniques and procedures is desirable but, for a variety of technical reasons, will be difficult to achieve (see Chapter 6). Furthermore, each political unit, be it city, county, state or federal government, may well have been studying the problem with little or no coordination with nearby political units. The result is invariably a fragmented approach which often makes comparisons impossible. The scientific variability of the background of the personnel frequently leads to one aspect of the study being emphasized at the expense of another. The State of California has attempted to overcome some of these problems by the establishment of guidelines for the various states' local agencies to follow (State Water Resources Control Board, 1972). The guidelines include discussion of the various biological, chemical, geological and physical parameters to be monitored and outline the procedures to follow in measuring each parameter. Thus far, it has not been possible to follow every guideline, but it has served and is serving as a goal. Many problems have been arising in the implementation of these guidelines; these include the lack of trained personnel, the lack of necessary funds to carry out the programme, or the reluctance on the part of some administrators to even approve the programme.

8.2.7 Sampling procedures

Localities to be monitored should be selected after the data on sources and types of contaminants have been compiled. Localities should be selected on the basis of sources of contaminants, including mouths of rivers. The number of stations within a locality should be selected to reflect not only the worst possible conditions but also have gradations to essential natural body burden levels. Localities should also be selected far from any source of pollution which would serve as a "control" or reference point. It is difficult to judge how many samples should be taken at a locality or at what frequency. It is important to recognize that in any monitoring problem continual evaluation should be made of the programme in order to ensure the monitoring programme does not become merely a data-gathering exercise. Initially, samples should be taken seasonally and at several stations within a specific locality. A thorough evaluation of all data should be made after one year to determine whether the station network or the frequency of sampling should be changed.

8.2.8 Coordination of participating laboratories

Coordination and communication of a study involving many laboratories located in many countries speaking different languages will probably be the most difficult problem to overcome. Periodic meetings must be held with a representative from each participating

laboratory. There are advantages to be gained by holding such meetings at each of the various laboratories. The primary purpose of these meetings is to ensure that all participants are aware of the progress of the entire project and to allow discussion of any problems which have arisen. A coordinator should be nominated whose responsibility is to oversee the entire monitoring programme. He should have adequate funds and staff to carry out the coordinating activities. It is important that he be able to travel to visit all laboratories involved in the study. The key to success during the preparational phase of the monitoring programme is thorough planning and adequate funding.

8.3 Screening Phase

The primary purpose of the screening phase of the monitoring programme is to ensure the equipment is functioning properly and that the technicians are trained. Intercalibration will constitute an important aspect of the screening phase. The studies outlined below should be conducted more-or-less concurrently.

8.3.1 Laboratory studies

The uptake and loss of contaminants by some of the organisms selected for residue analysis should be examined under laboratory conditions to obtain a better understanding of these processes. Specific body organs should be analysed whenever possible in order to assist in the interpretation of field data; studies should be conducted with some species in which changes in local environmental condition are reflected by alterations in the conditions of laboratory experiments. The various laboratories should cooperate in these laboratory studies which should include bioassays or toxicity tests. The primary value of bioassays in a monitoring programme lies in the interpretation of field residue studies, especially in determining whether or not the level of a contaminant in a field-collected specimen is at a critical level. It would be desirable to conduct some long-term bioassays through a reproductive period. However, apart from the biological and technical difficulties likely to be encountered in carrying out such tests, they may need to be of a long duration and are therefore likely to be expensive. Nevertheless, a knowledge of the effect of a contaminant upon those processes germain to an organism's population size and structure, would yield most useful information. Since these laboratory studies may require some time to complete, they need not necessarily be completed during the screening phase but could continue parallel to the operational phase.

8.3.2 Field studies

A small-scale pilot study should be undertaken by all laboratories for the primary purpose of testing all procedures. Each contaminant to be measured should be included, but only a few samples of organisms need to be collected for analysis. A meeting should be held after this pilot study for the purpose of discussing any problems which arose and possible solutions to these problems.

8.3.3 Evaluation

A thorough evaluation should be made by the director and personnel from each cooperating laboratory as to the progress of the entire study to date. Discussion should include such agenda items as the final selection of species, the number of samples to each species, the number of stations within a geographical area, the contaminants to be analysed at the different geographical localities, and any other problems which have arisen. Evaluation should also include the possibilities of successful completion of the project within the constraints of finances and personnel.

In summary, the successful completion of the screening phase is dependent upon communication with all laboratories involved. It is important that any equipment shortages or lack of trained personnel should be corrected at this time prior to the operational phase. Again, it is imperative that there should be sufficient funding to complete the task.

8.4 Operational Phase

The primary purpose of the operational phase is to put the pilot monitoring programme into operation. The operational phase should be run for a minimum of two years. The operational phase will be successful only if there has been adequate planning, coordination and funds.

8.4.1 Data collection and evaluation

All data should be transmitted to the central administration periodically so that evaluation can be continuous. It is important to ensure that all data are recorded in the same unit.

Data should be processed into the correct form for the computer as soon as they are received. Assessment of data in terms of scientific merit and cost factors should be a continuous process. Comparison of results between laboratories should be made periodically to make sure that the results are reasonable. Whenever the data for a particular contaminant seems abnormally high or low, these data should be carefully evaluated to ensure that: (1) the laboratory has not made an error, (2) the data should be compared to the input of pollutants in the locality for possible explanation, (3) whether data or other natural phenomena of the local region should be analysed for possible explanation, and (4) a sample of material should be analysed by another laboratory to see if similar results are obtained. Sufficient time and money should be made available at the end of the pilot programme to complete the evaluation of data.

8.4.2 Coordination and emergency procedures

As stressed in several places previously, it is imperative that there be sufficient communication of activities during the pilot programme. The coordinator should be kept fully informed of the progress from each contributing laboratory and he should keep each laboratory informed of the overall progress of the programme. Again, it is imperative that adequate funds be made available.

With many laboratories participating in the programme, it is reasonable to assume that one or more problems will arise at one or more laboratories with respect to a breakdown of equipment, lack of supplies, or a change in personnel. The coordinator should have sufficient power and resources available to make a quick decision as to what needs to be done. The coordinator will need to evaluate whether samples, for example, can be salvaged for analysis, if samples need to be analysed by another laboratory, etc., and which laboratory can carry out the necessary task. The solution of any problem will be easier if all planning and coordination has been thorough. The coordinator should have sufficient authority to make emergency decisions.

8.5 References

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9. ANALYTICAL CONSIDERATIONS

by

T. Yoshida

9.1 Introduction

Recent advances in analytical techniques have made it possible for scientists to determine the levels of contaminants that occur in the natural environment at extremely low concentrations, for example down to a few parts per trillion. However, analysis of such low concentrations can lead to serious mistakes and gross errors if the researcher performs an analysis without taking necessary precautions to prevent contamination during both the sampling and the analytical procedures. These dangers are amplified and compounded as the sensitivity of analytical instruments is improved, and more complicated purification procedures are adopted. In essence, the reliability of a set of analytical results is heavily dependent on whether the analysis is performed by well trained personnel and also whether the analytical method and especially the operation of the instruments has been conducted properly.

In order to achieve these objectives, it might be feasible to arrange for workshop courses to be originated and conducted by leading laboratories on a local or international basis in order to (a) properly train research personnel, (b) improve analytical techniques and (c) manage intercalibration programmes. If such courses could be organized, it would permit all analytical results to be evaluated with more confidence and on a broader basis than that which is possible at the present time, it would also pave the way for much needed cooperation.

9.2 Instrumentation and Analytical Procedures

Most of the analytical procedures which are likely to be used in the analyses of contaminants in organisms have been devised in the past decade and reflect the rapid advancement in electronic technology. At least initially, the analyst should follow well established procedures exactly as described. Examples of analytical procedures which can be used for a variety of contaminants in different substances are given in Part 1 of this Manual (FAO, 1975). Although provided methods used are carefully intercalibrated, there is no need to standardize methodology, indeed this may often be impracticable due to differences in instrumentation, changes in techniques should not be made part way through a programme unless the results by both techniques have been very carefully checked for comparability.

Intercalibration is, however, essential and this applies as much to changes of methods within a laboratory as to different methods used in different laboratories. It is worth noting in this connexion that even if it is feasible to standardize on a single method for use in all laboratories, this in itself does not guarantee comparable results. Also, standardization tends to stifle development and slows the adoption of simpler or better methods which may be developed during a programme.

The instrumentation required for modern analytical techniques is often quite advanced and, in many cases, alternative methods use different instrumentation. In some cases older, but often still valid, procedures are available using less sophisticated instrumentation; it should, however, be recognized that these techniques are often more time-consuming.

Examples of existing analytical procedures which are commonly used for the determination and quantitation of pollutants are summarized in Table I, which also shows the detection limit which can be achieved, the optimum sample size which is necessary for each analysis and analytical throughput which can be expected from a single operator per week in the

Table I

Examples of present analytical procedures for determination and quantitation of pollutants showing detection limits, optimum sample size and analytical throughput (samples/week • person)

Contaminant Category	Analytical Instrument	Detection Limits (g/g wet wt*)	Optimum Sample Size (g wet weight)	Analytical Throughput**
1. Heavy metals, inorganic				
Cd	Atomic absorption	3×10^{-9}	10	10-100
Pb	"	2.5×10^{-8}	10	10-100
Cu	"	3.5×10^{-9}	10	10-100
Zn	"	4×10^{-9}	10	10-100
V	"	1×10^{-7}	10	10-100
Ni	"	1×10^{-8}	10	10-100
Cr	"	1×10^{-8}	10	10-100
Mn	"	4×10^{-9}	10	10-100
As	(Flameless)	5×10^{-9}	10	10-100
Hg	"	5×10^{-10}	1	10-100
2. Heavy metals, organic				
Hg	GLC w/ECD	1×10^{-9}	10	10-40
As	TLC-AAS	1×10^{-8}	10	10-40
3. Pesticides and PCBs				
Chlorinated hydrocarbons	GLC w/ECD	1×10^{-9}-10^{-10}	10	5-20
4. Petroleum hydrocarbons				
n-Paraffin	GLC w/TLC	1×10^{-7}	10	5-10
Polynuclear aromatic hydrocarbons	HPLC w/GLC	5×10^{-7}	10	10-20

* Assuming a typical animal
** Assuming wet digestion, in the case of inorganic heavy metals

early stages of a monitoring operation. As the operator gains experience, throughput can be expected to increase. This table is not exhaustive and, as mentioned earlier, other methods are available although often these will have different detection limits. For example, neutron activation analysis, X-ray fluorescence, polarography, anodic stripping voltametry and even the classical colorimetric methods can be used for many metal analyses. Similarly, for petroleum hydrocarbons fluorescence spectrophotometry and even gravimetric techniques (using microbalances) can be used.

Since the measurement of radionuclides constitutes such a highly specialized analytical field, ecompassing a variety of techniques dependent on the nature of the radioactive decay, the resolution of the instrumentation and associated soft-ware, in addition to the different units of measurement, this type of contaminant has been omitted from Table I. The measurement of radionuclides was also not included in Part 1 of this Manual, and reference should be made to the section on analytical methods in Chapter 2.

Since instrumentation is still evolving, some improvements are still necessary and can be confidently expected. For instance, the near perfect separation of PCB from other chlorinated hydrocarbons might be achieved by high pressure liquid chromatography used in conjunction with GOMS computer systems. However, highly sophisticated instruments such as these are not always available in ordinary laboratories. Similarly, the problem of interference mostly attributed to molecular absorption in trace element analysis by atomic absorption in trace element analysis by atomic absorption have not been completely resolved. X-ray fluorescence spectrophotometry appears to show considerable promise in elemental analysis if its sensitivity can be improved.

9.3 Preparation of Reference Material

In order to achieve intercalibration of the various methods of chemical analysis and in order to ensure the comparability of resulting data, it is highly desirable that reference materials be prepared and distributed by authorized institutions. Much valuable work has already been accomplished in this area and many reference materials are available from a variety of sources e.g. NBS, IAEA. In the field of nutrient chemistry in the science of oceanography, intercalibration and comparison of data using identical samples independently analysed by a large number of institutions has been organized by ICES in cooperation with SCOR. Existing and potential substrates used as reference materials include dry powder forms of biological tissues such as fish and bivalve molluscs material, or phytoplankton cells. It is essential that the composition of reference material remains constant over a considerable period if it is to have any value. Water samples present special difficulties in this respect.

9.4 Statistical Analysis of Intercalibration

Cross-checking, which serves as a reliable index of accuracy of analytical methods and results, should be carried out prior to the start of the project work. Data from the analysis of reference materials may be processed statistically using a computer system to facilitate the evaluation. The following is a suggestion for the calculation of procedure, using the one way layout with unfixed repeat in the design of the experiment, the variation of obtained data both derived from the same laboratory (intra laboratory) and that from different laboratories (inter laboratory) can be defined as "within class variation" and "between class variation", respectively. First, the standard deviation for inter laboratory reproducability can be obtained from the following series of calculations:

Laboratories:	$L_1 \cdots L_i \cdots L_l$
Repetitions:	$n_1 \cdots n_i \cdots n_l$
Analytical values:	$x_i \cdots x_{ij} \cdots x_{in}$

$$\bar{x}_i = \left(\sum_{i=1}^{n} x_{ij}\right)/n$$

$$\bar{\bar{x}} = \left(\sum_{i=1}^{l}\sum_{j=1}^{n} x_{ij}\right)/ln$$

Intra-laboratory variance is given as

$$V_E = \frac{\sum_{i=1}^{l}\sum_{j=1}^{n}(x_{ij}-\bar{x}_i)^2}{l(n-1)}$$

while inter-laboratory variance is shown as

$$V_L = \frac{\sum_{i=1}^{l} n(\bar{x}_i-\bar{\bar{x}})^2}{l-1}$$

Standard deviation of intra-laboratory repetition

$$\hat{\sigma}_{w2} = \sqrt{V_E} \quad (\sigma_{Bl} \text{ for blank test})$$

Standard deviation for inter-laboratory reproducibility

$$\sqrt{\hat{\sigma}_{b2}^2 + \hat{\sigma}_{w2}^2} = \sqrt{\frac{(l-1)N}{N^2 - \sum_{i=1}^{l} n_i^2}}\, V_L + \left\{1 - \frac{(l-1)N}{N^2 - \sum_{i=1}^{l} n_i^2}\right\} V_E$$

And then CV (%) which indicate the relative dispersion of each standard deviation is obtained as

$$(\hat{\sigma}/x) \times 100 = CV\ (\%)$$

Finally, the inter-laboratory reproducibility tolerance (precision) is given as

$$D_2(0.95)CV_i = D_2(0.95)\sqrt{\hat{\sigma}_{b2}^2 + \hat{\sigma}_{w2}^2}\Big/\bar{\bar{x}}$$

where $D_2(0.95) = 2.77$ $(l = 2)$.

As an example of the sort of variations which may occur, this technique was used to evaluate the results obtained by five laboratories for total mercury and methyl mercury analysis. The results of this evaluation are shown in Tables II and III and Figure 1. As a further example, data from the literature on the precision of mercury analysis of water samples are shown in Table IV. These examples show clearly that the degree of precision achieved varies with the substrate analysed but that, regardless of the substrate, precision deteriorates as the level to be determined is reduced.

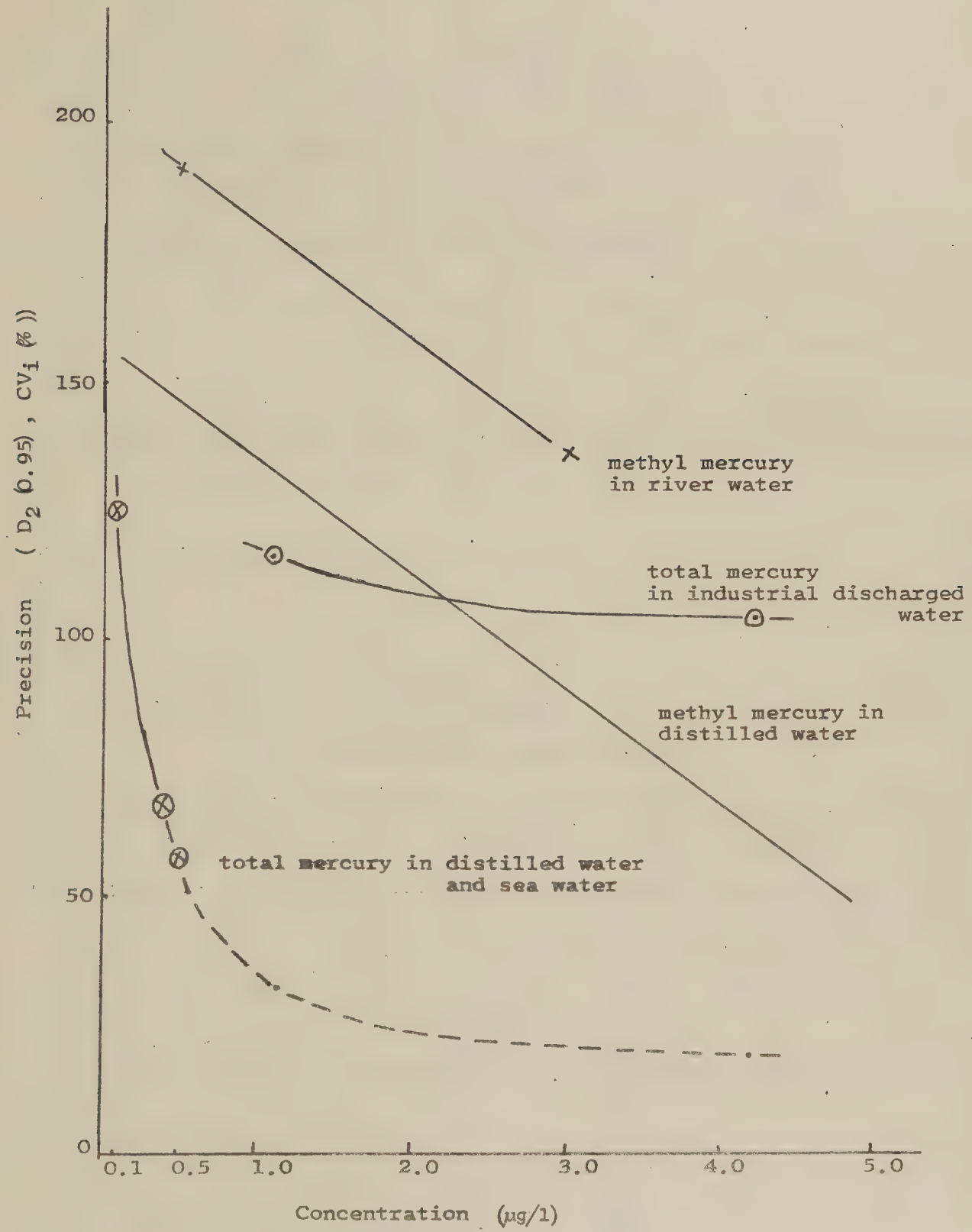

Figure 1. Precision of total and methyl mercury analysis

Table II

Precision of total mercury analysis

Sample	Concentration µg/l	Precision $D_2(0.95)$, CV_i (%)
Sea water	0.1	125
	0.5	58
Distilled water	0.4	70
Industrial discharged water	1.1	116
	4.2	105

Table III

Precision of methyl mercury analysis

Sample	Concentration µg/l	Precision $D_2(0.95)$, CV_i (%)
River water	3.0	139
	0.5	187
Distilled water	0.1	150
	1.0	145
	5.0	45

Table IV

Precision of mercury analysis in water samples

Literature	Method	Sample	CV (level µg/l)
Kalb, 1970	Reduction	River water	5.7% (1.06), 3.0% (10.0), 1.2% (2.48)
Doherty and Dorsett, 1971	Reduction	Envir. water	10% (0.1-10)
Umezaki' and Iwamoto, 1971	Reduction	Std. soln	1.7% (5), 1.1% (5, w/alkali method), 2.0% (5, MIC)
Stainton, 1971	Reduction (equilibrium method)	Std. soln	2.11% (4), 1.19% (8), 1.19% (12), 0.63% (16), 0.54% (20)
Environmental Protection Agency, 1971	Reduction	River water	46% (0.35), 10.3% (1.35), 30% (3.35), 1.8% (4.35)
	Deposit on Ag	Std. soln	31% (0.13), 11% (1.88), 8.1% (88)
Sagami Chemical Research Center (personal communication)	Deposit on Ag	Sea water Lake water	10% (8), 10% (10), 16% (6)

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